

29 October 1981

Conflicts of interest about development

The Cancun summit has proved to be a modest success after all, but the hard talking has not yet begun

Politicians returned from last week's conference on development at Cancun in Mexico have been boasting that the occasion was less quarrelsome than had been feared. Certainly no delegation walked out. Recriminations (not entirely absent) may have been restrained by the complainants' wish that there should be further talks on economic relations between industrial and developing nations. The developing nations seem also to have wrung concessions from the industrial states (see page 691). Perhaps the chief of these was the presence of President Ronald Reagan and his entourage — President Carter had been unable to decide whether turning up would do more harm than good. In the event, the Cancun conference turned out to be a modest success. So has a substantial beginning now been made on the problems of development and on the ambitious agenda sketched in the report of the Brandt commission nearly two years ago? Last week's conference will have helped a little, but the nub of the problems of development remains largely untouched. There is a great deal of talking, some of it recriminatory, still to be done.

Agriculture, the least contentious of development issues, is a good illustration of the deep differences of opinion and conflicts of interest that persist between developing and industrial states. Everybody, of course, agrees that a substantial and continuing increase of food production is urgently necessary in many developing countries. How is this to be accomplished? The people at Cancun appear to have agreed that there should be a reexamination of the machinery of the United Nations organizations concerned with agricultural development. This is a sensible decision. Different kinds of assistance with agricultural development are at present channelled through too many agencies. Technical assistance, for example, may be had from the Food and Agriculture Organization (FAO), the World Bank, the United Nations Development Programme (UNDP) and through bilateral agreements between the governments of developing and industrial states. Grants and loans for long-term development are provided by the World Bank, UNDP and again by bilateral agreements. Food may be scarce in developing countries, but there is probably a glut of external technical advice. So why are so many developing countries still unable to feed themselves? And why do industrial states so often drag their feet in contributing to the scale of international assistance for agricultural development?

A large part of the problem, not often openly discussed, is that the governments of developing countries have often failed to give agriculture the priority it deserves in the whole process of development. In many places, the retail prices of basic foodstuffs are rigidly and uneconomically controlled. Low food prices may suit urban populations (where votes are to be found at election time) but also discourage farmers from growing as much food as they might (or persuade them to sell it only on the black market). India is lucky to have moved to self-sufficiency while operating such a system. Elsewhere (Ethiopia, for example) farmers may be compelled to sell their produce only to state-run marketing organizations whose effectiveness and even honesty is questionable. Other governments (Tanzania?) have subordinated food production to other social goals, collective land ownership for example. Other developing governments have lavished their attention on exportable cash crops to the neglect of domestic food production. It is in this connection absurd that Ghana should be better able to supply the children of the industrial world with the ingredients of their chocolate bars than its own people with basic foodstuffs. A more cruel irony is that food production in the

developing world should be as patchy as it is.

Well-wishers of development must therefore acknowledge that as in many of their own industrial countries, the application of known and beneficent technology is frequently impeded by governments bent on squaring political circles of some kind. To concede this is not to overlook what has been accomplished in many developing states — India is merely the most conspicuous example not to ignore the serious physical and climatic impediments to agricultural development that persist in many places, the countries of the Sahel region for example. The consequence, however, is that resentment must arise in the relationship between the many governments unable to feed their people and those elsewhere which might be able to help. Unfortunately, these conflicts are too often hidden behind euphemisms such as "let those in need do more to help themselves". Mr Reagan's advice that developing countries should do more "to pull themselves up by their bootstraps" is a less tactful version. The underlying conflict is that between the industrial states protesting that the governments of developing countries waywardly make ineffectual use of the help available and the putative recipients which complain that aid is more dependent on unwanted political interference. The conflict is real, and will not go away unless people talk about it openly — and more formally than at Cancun.

Similar conflicts arise in other fields, energy for example. The developing countries, at the United Nations conference on the subject in Nairobi last August and now in the more select gathering at Cancun, have been looking for two kinds of help — research and development that might contribute towards self-sufficiency and, more immediately, financial assistance with which to buy oil. Here again, however, the existing technology of energy production (and that likely to be developed in the next few years) is technically a sufficient basis for increasing energy production in developing countries. More use would have been made of it if organizations such as the World Bank had had more funds to lend in recent years, and if the governments of developing countries had been more willing to make equitable agreements with the organizations that have become the chief agents of international technology transfer — the much reviled multinational companies skilled at drilling for oil, building dams or even nuclear power stations. (The complaint that the same companies have often in the past been exploitative has substance, but is also easily avoidable by simple legal instruments: the complaint that they seek to make a profit on their operations is irrelevant.) So the proposal canvassed in Nairobi in August that there should be a new international agency for the development of energy resources "appropriate" to developing countries is both a diversion and a sign to others that the governments of developing countries may not all have appreciated the seriousness of the problems that afflict them.

For many developing countries, however, the financial consequences of more expensive oil are more immediate than physical shortages. Since 1974, for example, the economic prospects for Tanzania have been transformed from hope to crisis. For the governments concerned, it is especially galling that they have felt the full effects of dear oil while these have been partially avoided or at least postponed in industrial states because the oil producers obligingly return a substantial part of their financial surpluses by direct investment or even by lending directly to the governments of the industrialized states. (Part of



the price of this convenience is rapid inflation now and a load of unrequited debt for later.) So should the international monetary institutions provide loans to help developing countries out of immediate trouble? The United States has stoutly resisted this proposal, and rightly. In the long run, the cause of development itself would be undermined if the World Bank and the International Monetary Fund were saddled with loans that could never be repaid. The best solution is that the international institutions should stick to their rigorous practice of not lending on empty prospectuses but that they should also be better equipped to finance economically sound oil-substitution schemes in developing countries. The question of principle that remains to be hammered out is whether the developing countries will accept the terms on which such assistance is offered.

Policies and prejudices inimical to development are not, however, confined to the developing countries, whose strongest argument is that industrial states seeking to bolster up a declining domestic textile or footwear industry habitually make it harder for them to pull themselves up by their bootstraps. Yet the best hope for both industrial and developing states is that the division of labour between them will find its natural (and constantly changing) level. This, so far as it goes, is the only durable basis for the "new economic order" on which many developing countries have set their hearts. The snag, for both parties to such a deal, is that the objective of industrial self-sufficiency must be replaced by that of interdependence. The domestic political and economic problems arising from an understanding along these lines would be serious. But no purpose will be served by hiding from them. If, as agreed at Cancun last week, there are to be talks within the United Nations on "global" economic problems, it is essential that the difficulties should not be buried. For it is a continuing frustration that the potential benefits of available technology should be denied because of the unwillingness of normally over-talkative politicians to say what is on their minds.

Three million jobless

The growing army of the British unemployed may one day have jobs again. But what? And how?

With British unemployment now statistically indistinguishable from three million, or more than eleven per cent of the workforce, it is natural that the government should be nervous, the unemployed angry and those still with jobs both anxious and confused. The dole queues will be even longer by the time that the new Technical Change Centre has something to say on the subject which is central to its programme (see page 695). Already the rolls of the unemployed have been swollen by several thousand teachers from the schools, where demography of necessity strikes first. In the next few months, they will be further increased by some thousands of academics from higher education — some from the universities, some from the other institutions of higher education once the government has learned to cut them down to size. So what is to be made of it all?

The simplest and most comforting assumption is that the dole queues will shrink again when prosperity returns, whereupon people will go back to the jobs from which they have recently been displaced. But that implies that the upheavals of the past few years will have left no lasting mark on the pattern of British industry. The more disturbing but more realistic assumption is that the lists of the unemployed presage a lasting change similar in kind that required to strike an accommodation between the rich and poor countries (see above) but driven by the new technology whose seeds now abound (see *Nature* 15 October, p.499). Already, in Britain and the rest of Western Europe, steel-making capacity has been reduced and is unlikely ever again to be increased. If the demand for steel picks up, new plants elsewhere will be able to take up the slack more cheaply. The shift from manufacturing industry to service industries, the twentieth century equivalent of the previous century's drift from the land, will continue. In the years ahead, part but not all of the service sector will need fewer

people to do the same work, with the result that during the recovery from the slump, it will be hard to tell whether people will be displaced more rapidly by automation from manufacturing industry than by administrative computers from the office. So how, it will be asked, is it possible to plan ahead?

The simple answer is that planning ahead is not possible at all: the future for jobs, in Britain or anywhere else, will depend on the success with which countries are able to compete in productivity. But one thing is clear. The valuable jobs will be jobs that require technical skill. Not merely will they reward those who do them more handsomely than other jobs, but they will contribute substantially to the wealth of the community. In an ideal world, the two components of the job queues — the teachers displaced from their jobs and the young people who all too often have tragically not had a chance to work — would pool their resources so as to make the best of this circumstance. The teachers would help the unskilled young people to become eligible for the kinds of jobs the future needs. They might even in the process decide to establish some kind of institution for the transfer of skill from the skilled to the unskilled jobless. They might even call it a university. If they did so, the Privy Council would probably be asked by the University Grants Committee to stop them.

So must not this be the worst time for the British government to cut back the budget for education? That is the obvious conclusion. Yet it is too obvious. For the British government has no choice but to save money across the board. Numerically, education has not done badly in recent years. The abruptness of the cut in the universities will harm institutions and scholarship principally because it is so abrupt — if the universities had been given say five years (not three) to live with 8.5 per cent less each year, and had been allowed to be flexible both in the pattern of their teaching and the means by which they recruited funds, few of them would have been able to complain. It is not, after all, as if the British educational system is above reproach. At all levels, it is academically too specialized, too snobbish (Oxbridge) and too slow to change. The complaint that academics should be making against the government is that it has not merely cut the public funds available but also settled the scale and pattern on which educational institutions will operate for years to come. Apparently persuaded that the educational system as it is could not cater for the future need of skill, it has neglected to ask whether a differently organized system might serve the purpose better. If it persists in this indifference, there is only a meagre chance that unemployment will shrink below three million.

The unemployed also have a complaint to make more subtle than that so far made on their behalf by the trades unions. If the present upheaval in British industry marks a permanent and structural change in the pattern of industry, those now unemployed (and their children) have a right to ask for a chance of preparing for the new regime. Although the present government has continued many of its predecessors' schemes for retraining people changing jobs, these opportunities are designed to produce traditional craftsmen, bricklayers and carpenters for example, not the kinds of skilled people likely to be needed in the years ahead. Similarly, the youth opportunities programme which services to keep more than half a million school leavers off the streets (and the unemployment roll) is more a device for allowing young people to learn at first hand what the now obsolete skills are used for than a preparation for the future. Is it too much to hope that the new Secretary of State for Employment, Mr Norman Tebbit, will recognize that this practice is absurd? What he must devise are ways of helping young people to be trained as computer programmers, as people who know what electronic equipment is like, as people who may, when the economy picks up, have to use numerically controlled machine tools. Several steps are needed. The costs of fees paid to educational institutions (private as well as public) should be deductible from a person's income before he pays tax. The public sector of the educational system should be made more flexible, able to take in people without formal academic qualifications, in the evenings. And the government itself should provide those in the dole queues with a more realistic account of what the future may be like.

Hopes from the North-South summit

Third World struggles for UN changes

Cancun, Mexico

Last week's meeting of the heads of state of 22 developed and developing nations in Cancun could help to break the stalemate which has characterized recent efforts to devise new strategies for technical and financial assistance to Third World nations through agencies of the United Nations.

The meeting failed to provide the clear resolution which many had hoped for. In summing up the discussion, the co-chairmen, Mexican President José Lopez Portillo and Canadian Prime Minister Pierre Trudeau, stated merely that there had been agreement to support preparations within the United Nations towards "global negotiations", the term being used to embrace efforts to overhaul the technical and financial agencies of the United Nations for the greater benefit of the Third World with no clear indication of when or how any changes would be implemented.

This failure to reach specific agreement reflected the opposition, particularly from the United States and the United Kingdom, to any attempt at moving decision-making responsibility for the financial agencies of the United Nations into the UN General Assembly, where they would all become subject to a one-country one-vote system of control. However, delegates attending the Cancun meeting expressed cautious optimism that it had at least provided a basis for more detailed discussions.

It had been hoped that two areas concerned with the applications of technology to development would receive a significant boost from the Cancun meeting. First, the World Bank's efforts to increase the amount of capital available for investment in energy projects in Third World countries. And second, the continued attempt to put into effect the recommendations of the UN Conference on Science and Technology for Development which took place in Vienna in 1979.

There was some slight but significant movement on both these fronts. The idea of an energy affiliate attached to the World Bank, thought to have been virtually scuppered by the opposition of the Reagan Administration, is far from dead. Many heads of state at Cancun spoke strongly in favour of the proposed affiliate. Particularly significant was the announcement by Saudi Arabia that it too supported the proposal. To the United States this seemed to imply the creation of a new "energy sector" to finance energy projects within

the World Bank, although the United States and the United Kingdom insist that this should only be done if there were new funds for the purpose.

At the same time, members of several delegations informally expressed support for a new initiative, now backed by 20 developing countries which signed a declaration at a meeting in Caracas three weeks ago, to set up a special fund for helping developing countries to build up their scientific and technological infrastructure.

These funds would be used to carry out some of the recommendations of the Vienna meeting, with a suggested initial target for voluntary contributions of a million million dollars over the years 1982-87. One possible form for the fund would be to base it on the International Fund for Agricultural Development (IFAD), set up after the World Food Conference of 1972 with funding from the developed nations, the oil-producing nations and the developing nations.

But the differences that remain, both on the appropriate way of raising capital for energy projects in the Third World and on

the best method of building up their scientific and technological infrastructure, reflect the wide gulf between the free market ideology of the United States and the interventionist stance of most Third World governments.

On food, for example, President Reagan had announced previously in a speech in Philadelphia that the United States intended to shift its aid efforts towards market-oriented qualities that would create "self-sustaining capacity" for research and innovation, and stimulation of job-creating entrepreneurship in rural areas.

Those attending the Cancun meeting agreed that priority should be given to finding ways in which developing countries could raise their own food production rather than to providing direct food aid.

But several developing countries also pushed for more direct intervention, for example, in the setting up of large emergency food stockpiles to compensate for poor harvests, and for greater efforts to establish a world food system embracing both production and distribution.

Discussions on both energy and food

Monkey business hits university laboratory

One of Britain's largest and best laboratory breeding colonies of rhesus monkeys is finally to be disbanded despite the fact that it has not been possible to import the animals from their usual source for the past three years. About half the monkeys are likely to find homes elsewhere but the other half may be killed — ten of them already have been — because they harbour a virus which can be fatal to man.

The virus is herpes simian B virus, with which monkeys can be either acutely or latently infected. An animal in the former state is very dangerous since the virus can be passed on to man through a bite; most of the recorded human infections of this type have been fatal. By contrast, animals which carry the virus in latent form pose no threat to humans except for the possibility of reactivation of the virus to its infectious form in conditions of severe stress. That possibility seems remote to judge by the lack of concern that many of the monkeys in zoos and wild life parks carry the virus in latent form.

The University of Birmingham, however, became particularly concerned about its monkeys in the wake of the death in 1978 of one of its staff from smallpox contracted, it is believed, from a virus that had escaped from one of their laboratories. That incident, the fact that the Medical Research Council had just issued new guidelines on the hazards of simian virus and the general state and age of the housing for their rhesus monkey colony, led the University of Birmingham to ask the Health and Safety Executive to give advice on the safety standards of the monkey

housing. The upshot was that the university decided that it would cost £250,000 to modernize the building — a figure believed in some quarters to have been wildly inflated. In any case the university decided it could not afford modernization and that the colony would have to go.

That decision was made in September 1980 (although work on the monkeys carrying latent virus had been suspended in February of the previous year for lack of adequate safety). Following the decision Dr J. Marston, a qualified veterinary surgeon who had been closely involved with the maintenance of the colony for several years, suggested that he should be allowed to remove the monkeys to private housing and breeding facilities which he hoped to establish. The university agreed in principle but, after considerable capital had been raised, Dr Marston's plans were thwarted by the Home Office which was not satisfied with the proposed facilities. According to Dr Marston, the Home Office moved very slowly to its final decision and three weeks before it was finalized, at the end of September, the university threatened to start killing off the monkeys. Two weeks after the decision, on 12 October, the university ordered the killing of the infected animals at the rate of ten per day. Ten were killed with the participation, at his own insistence, of Dr Marston but a stay of execution was then issued. Dr Marston is now trying to find homes for all the monkeys including those with the latent virus, Home Office rules permitting.

Peter Newmark

were generally thought to have moved forward at Cancun, perhaps providing a basis for later, more substantial, agreements. But the movement which several national heads such as Mrs Thatcher and French President Francois Mitterrand claimed was made in the direction of the "global negotiations" being demanded by the more militant of the developing nations was so small as to be virtually imperceptible.

David Dickson

German cancer centre

Commission meets

The first meeting has taken place of the International Commission appointed to look into the continuing problems of the German Cancer Research Centre in Heidelberg (see *Nature* 1 October, p.328). The commission spent 21–23 October in Heidelberg talking to all parties concerned and expects to complete its report in mid-December for delivery by the end of the year.

The task of the commission, under the chairmanship of Sir Michael Stoker, is to carry out an impartial investigation of the problems of governance of the centre and the quality of its research programme following the resignation in June of the director, Dr Hans Neurath. On 23 September Dr Neurath made clear his case saying that his plan to improve the quality of research at the centre had been obstructed and that the institution was "plagued by serious and perhaps irreconcilable conflicts of interest", largely stemming from an "old established majority".

While in Heidelberg the commission met, among others, Dr Neurath, the heads and/or staff representatives of all eight of the institutes that make up the centre and Dr Wolfgang Finke, chairman of the centre's supervisory body, the Kuratorium. The commission also reviewed recent publications by the staff of the centre.

The commission is likely to focus its attention on the Scientific Council of the centre, an internal body consisting of the eight heads of institute together with one other member of each institute who is elected by the staff. As it stands, any director of the centre wishing to make major changes in its activities would need either to have the majority support of the Scientific Council or be prepared to try and push ahead regardless. It is clear that Dr Neurath's proposals to bolster the quality and quantity of basic research at the expense of some clinical therapy and anti-cancer drug screening did not enjoy the support of the Scientific Council.

It will be for the next director, not the commission, to decide what policy to implement. He or she is not likely to be chosen until after the commission has reported but an announcement of the interim director is due in mid-November. The commission must hope that its advice leads to the possibility of effective direction next time round.

Peter Newmark

New improved PWRs

The task force appointed during the summer to streamline the design of a pressurized water reactor (PWR) suitable for British conditions has worked swiftly. Its new reference design was adopted by the Central Electricity Generating Board last week. The National Nuclear Corporation, for whom the task force did its work, will now draw up detailed specifications based on the reference design for Britain's first PWR planned to be built at Sizewell in Suffolk. The detailed design should be published next April or May, but the corporation plans to have an estimate of cost within two months.

The task force's apparent success follows on the nuclear corporation's earlier failure to design a satisfactory British PWR. The corporation's efforts were scrapped by the Department of Energy after the estimated cost of the reactor began to soar, largely due to the incorporation of extra safety features. The department appointed Dr Walter Marshall, chairman of the UK Atomic Energy Authority, to lead the task force to come up with a cheaper design quickly.

The details of the new reference design have not been released, but it is thought to resemble closely the standardized nuclear power plant system, SNUPPS, on which PWRs built by Westinghouse in the United States are based. It is unlikely that it retains any of the extra safety features incorporated in the previous attempted design, which included extra emergency core cooling systems, modified coolant pumps and extra thick concrete shielding.

The task force's design has taken only three months. Speed was important to meet the deadline set for the public inquiry into the siting of the reactor at Sizewell due by the end of 1982. A detailed specification of the reactor must be published well before the inquiry to permit public scrutiny.

Judy Redfearn

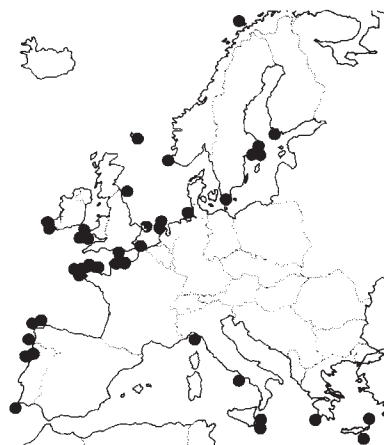
Oil pollution

British problems

The British government machinery for coping with major oil spills is inadequate, according to the Royal Commission on Environmental Pollution, whose latest report on oil pollution of the sea was published last week. The problem is that too many government departments, local and other interested bodies, often with conflicting interests, are involved in clean-up operations. The commission's report recommends that responsibility for dealing with the larger spills should be vested solely with the Marine Pollution Control Unit, which should be strengthened to cope with the task and made responsible to both the

Departments of Trade and the Environment.

The commission's report is the outcome of three years of study begun when the *Amoco Cadiz* spilt 200,000 tons of crude oil after running aground in the English Channel off the north coast of France. Although British coasts escaped extensive pollution (the coasts of Brittany bore the brunt), the incident reinforced public anxiety over the environmental consequences of major oil spills. The then Labour government requested the royal commission to investigate the general issue and accordingly its report is wide ranging, including sources of oil pollution, the consequences of both chronic pollution and major spills for marine life and public amenities and international and national attempts at prevention and control.



Sites of major oil spills around Europe

The report, however, has one word of reassurance. The commission found that neither chronic oil pollution nor major spills pose a permanent threat to the marine environment. Oil slicks affect marine life only for as far as they extend and as long as they last. Once they disperse, marine systems usually recover over a period of a few months to several years. Although sea birds are more susceptible to the effects of both chronic pollution and major spills than are fish, the commission found no evidence that any breeding colonies in Britain are currently threatened. Intertidal ecosystems are likely to suffer more than marine life in the open sea.

The commission found that research on the effects of oil on marine animals is adequate but that a lack of knowledge of the natural variability of marine systems makes it difficult to assess the singular effects of oil spills. Hence, rather than more research it urges greater coordination, in particular between research on oil and that on other pollutants such as heavy metals and polychlorinated biphenyls.

Despite the reassurance, however, the commission says that the immediate effects of major oil spills are sufficiently unpleasant to warrant greater effort at prevention and a more efficient way of coping with accidents. British government policy comes in for a drubbing. According to the

report, the Department of Trade lacks a sense of urgency in negotiating international agreements on standards for oil tankers and controls to monitor the competence of crews. The hydrographic survey of shipping lanes around Britain is also inadequate. The report recommends that the government should extend the limit of territorial waters from three to twelve miles and that permission for oil drilling in areas of the North Sea designated environmentally important should be refused.

The brunt of the criticism, however, is reserved for contingency plans to cope with oil spills. Apart from divided responsibilities, the commission criticizes the emphasis put on the use of chemicals — themselves more dangerous to marine life than oil — for dispersing oil slicks on the open sea. More attention, it says, should be paid to coping with oil in harbours and on the sea shore. Local authorities should remain responsible for minor spills, especially those inshore, but a small permanent core of experts should be available nationally to coordinate clean-up operations and advise on major spills.

The commission rejects the government's proposal to set up local Coastal Pollution Coordination Centres when major spills occur, on the grounds that their remit to achieve consensus between interested bodies will render them ineffective. Instead, it recommends that the existing Marine Pollution Control Unit, responsible to the Department of Trade, should be revamped to take on the task. The unit's operations at sea should remain under the trade department but operations onshore should come under the responsibility of the Department of the Environment.

Judy Redfearn

Indian satellites

November launch

New Delhi

India is hoping to launch its second Earth observation satellite, Bhaskara II, some time in November before it takes a major step in space communication technology when Indian National Satellite IA (INSAT IA) is launched in April 1982. Bhaskara II, an improved version of Bhaskara I, is now being transported to the cosmodrome at Volgograd in the Soviet Union for installation on board a Soviet launch vehicle.

Bhaskara I, launched on 7 June 1979, was expected to function for a year, but continued to operate normally until 1 August this year. The satellite achieved most of its goals in the process. Bhaskara II is to carry a two-band television camera for visible and near infrared imaging and a three-frequency radio meter operating at 19,22 and 31 GHz frequencies.

Meanwhile, INSAT IA is being prepared for launch on a Delta vehicle from Cape Canaveral in the United States. INSAT IA is a three-axis stabilized satellite

like Apple, India's experimental communications satellite launched last June. INSAT IA is to be stationed in a parking orbit at longitude 74°E; this will facilitate nationwide television and radio broadcasting and long distance telephone connections during its seven years in orbit.

India's meteorological department is setting up a meteorological data utilization centre in Delhi, 110 data collection platforms on land and sea all over the country and 100 disaster warning sets in the coastal areas to operate in conjunction with the meteorological payloads to be provided by INSAT-IA. The telecommunications component will provide more than 8,000 two-way long distance telephone circuits, round-the-clock weather forecasting and mapping of the entire country.

INSAT-IA will be followed by INSAT-IB to be parked in the 94°E geostationary orbit.

The International Telecommunications Satellite Organization has agreed to increase by 12 per cent the capacities of the two Indian satellites to be placed in the geostationary equatorial orbit. This would make possible more intensive use of the telecommunications capacity of INSAT.

Sunil Saraf

Sun-comet collision

Extreme conditions

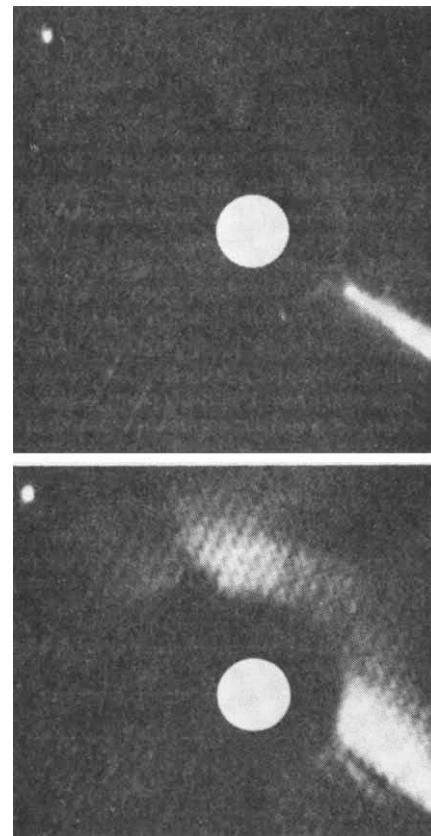
The first ever recorded collision between a comet and the Sun was detected by a US Air Force satellite in mid-1979, the Department of Defense has revealed. The news has only just emerged because the satellite experiments had been given low priority, and the data tapes have only just been analysed.

Dr Donald J. Michels, the Naval Research Laboratory scientist whose "Solwind" coronagraph detected the collision, estimates that the impact velocity was 640,000 miles per hour. But the orbit is not very well determined because the comet only appears on eight frames, covering 138 minutes, before it disappears behind the occulting dish of the coronagraph. A few hours later, a gigantic cloud of debris is visible extending a few million miles above the solar surface. The record continues for about 24 hours after the collision.

Michels' experiment was not designed to detect comets, but to collect data on coronal transients which might later be correlated with magnetic storms or aurora on Earth — phenomena which can interfere with missile warning systems. It consists of a one-inch telescope designed to give high rejection of scattered light. It is aimed at the Sun, with an occulting dish shadowing a region of about two and a half times the solar radius. Thus the apparatus did not detect the actual moment of contact on 30 August 1979, but only the approach of the comet and the coronal events after the collision.

The telescope — still in operation — records a white light image on a videocon tube, so there is no spectral information. But it is possible to extract some polarization information, Michels says.

Michels is not a cometary scientist, and to analyse the data he will work with Dr Zdenek Sekanina, an expert on cometary dust at the Jet Propulsion Laboratory, Pasadena.



A Sun-grazer comet collides with the Sun on 30 August 1979, observed by a US defence satellite. Above: the approach. Below: the debris. The Sun is obscured by an occulting dish to the equivalent of 2.5 solar radii, but the white disk indicates the Sun's position and size. Venus appears to left. Eleven hours separate the two pictures.

According to Sekanina, the most important missing factor is the cometary orbit. Without that it is difficult to analyse the three-dimensional motion of the dust and tail. So Michels has put out a request for other observers to check their data to see if there are any earlier pictures of the comet on the way to the Sun. So far they have had no success.

But even without the orbit it will be possible to learn something from the pictures, says Sekanina. The time evolution of the tail in the intense radiation environment close to the Sun should yield new data on dust particle sizes and composition. For instance, it would be interesting to estimate the distance from the Sun at which the particles start evaporating.

The collision is an extreme case, in extreme conditions, says Sekanina, and that is bound to reveal something.

Robert Walgate

Greek politics

Cultured science

The success of the new socialist government in Greece under Mr Andreas Papandreou seemed to have introduced a touch of rare glamour to science politics: the actress Melina Mercouri was to have been Minister for Culture and Science. Unfortunately the "science" in her title is merely a tag, and Miss Mercouri's role will be more to do with monuments than molecular biology.

Affairs more directly affecting scientists are likely to rest with the Minister of Economic Coordination (Mr Apostolos Lazaris) and the Minister of Education and Religious Affairs (Mr Leftheris Verivakis). Lazaris will master the three-year-old Science, Research and Technology Agency, which is reorganizing basic science funding along the usual western lines of a research council; and Verivakis will direct the reform of the 14 university-level institutions of Greece.

If its election campaign promises are to be believed the new government will show less interest than the previous one in a (hypothetical) Greek nuclear power programme. Recent earthquakes have increased popular resistance to nuclear energy (for fear of damage to reactors), and Papandreou has called for "more research".



President Caramanlis meets Minister Mercouri

Papandreou, for 20 years a professor of economics at Harvard University, is also interested in university reform, and is likely to encourage Verivakis (a 40-year-old lawyer) to move rapidly to implement his party's proposals. These broadly follow the lines of the reform committee, headed by Professor F. Mitsis, which reported to the last government. Verivakis, though, may go further in proposing open entry to the universities and the abandonment of the present entrance examination.

Verivakis may also attempt to increase the numbers of postgraduate students in Greece — something that should follow the substitution of the paternalistic "chair" system by a more modern departmental organization for the universities.

Opening the universities to all comers, however, may have to wait until the Greek economy picks up. For example, in the 1982 entrance examinations, just finished, there were 82,240 candidates for 26,694 places.

Robert Walgate

Scientists against nuclear weapons — a list

Readers — particularly those who do not realize that 24–31 October is United Nations Nuclear Disarmament Week — may be interested to know of the following organizations which have been set up by and for scientists who are opposed to nuclear armaments. The self-expressed objectives of the organizations are, generally speaking, to coordinate and enhance technically informed opinion, inside and outside government, concerning nuclear armaments policies. The expanding membership of these organizations reflects a growth of concern in the scientific community, illustrated also by a statement signed by at least half of those attending the recent International School of Plasma Physics at Varenna (though not by the Soviet or Chinese participants). The statement emphasizes that "physicists bear a heavy burden of responsibility for the present danger", and that they should "oppose the resignation and passivity which is so widespread". The following list is not exhaustive and we would like to be informed of other organizations around the world formed for the same purpose.

● Union of Concerned Scientists, 1384 Massachusetts Avenue, Cambridge, Massachusetts 02238, USA.

● Ground Zero, 806 15th Street, Suite 421, Washington, DC 20005, USA.

● Physicians for Social Responsibility, 25 Main Street, PO Box 144, Watertown, Massachusetts 02172, USA.

● International Physicians for Prevention of Nuclear War, 635 Huntington Avenue, Boston, Massachusetts 02115, USA.

● Science for Peace, c/o Professor D. Paul, Department of Physics, University of Toronto, Toronto M5S 1A7, Ontario, Canada.

● Canadian Peace Research Institute, Gryffin Lodge, Huntsville POA 1K0, Ontario, Canada.

● Scientists Against Nuclear Arms, 11 Chapel Street, Woburn Sands, Milton Keynes MK17 8PG, UK.

● Medical Campaign Against Nuclear Weapons, 120 Edith Road, London W14, UK.

● The Pugwash Conference on Science and World Affairs is not a "membership organization" in that people attend the annual conference by invitation. Individuals may, however, join the national branches of the organization to be directly informed of its activity.

Philip Campbell

US telecommunications

End to AT&T saga?

Washington

In a move which could persuade the Reagan Administration to drop anti-trust charges against the massive American Telephone and Telegraph Company (AT&T, otherwise known as the Bell System), the US Senate has passed legislation that would allow the company to set up a wholly-owned subsidiary to enter the computer and information processing fields.

Similar legislation is expected to be passed soon by the House of Representatives, and a deregulation bill is likely to be signed into law by President Reagan early next year. The bill marks an attempt to bring US telecommunications into line with the technology of the 1980s and is the first major change in structure of federal controls since the 1934 Communications Act.

Congress has been struggling for six years over what to do about AT&T. Under a consent decree reached with the Department of Justice in 1956, AT&T — the largest company in the world, with assets exceeding \$125,000 million — was granted a virtual monopoly in the regulated local and long-term telephone markets, but on condition that it did not enter any unregulated markets.

The latter category includes computer equipment and data processing. As these fields have blossomed in recent years, so has AT&T's desire to enter and compete in them — and therefore to change the terms of the consent decree. This it would now be able to do under the legislation approved by the Senate, through a subsidiary generally known as Baby Bell.

Responding to criticisms that, if the new company and its parent were too closely linked, its degree of support would constitute unfair competition against other companies already in the information processing field, the Senate has stipulated that the two should have an "arms-length" relationship. This is one of the conditions that the Justice Department has decreed must be included in any deregulation legislation, if it is to drop its anti-trust suit against the company. That suit, begun six months ago, remains adjourned.

More concessions are expected to be demanded in the House, reflecting the views of AT&T's largest corporate subscribers. Many are alarmed, for example, that Baby Bell might establish its own more sophisticated long-distance telecommunications system to compete with the now ageing Bell System, but without the local controls to which the current Bell System, which carries 98 per cent of all long-distance voice and data transmission, is subject.

David Dickson

Technical innovation

All change

Britain's newest think-tank, the Technical Change Centre, appears to be limbering up for thought. Sir Bruce Williams, director of the centre, announced recently the appointment of two assistant directors at the establishment, Professor Ronald Dore of the University of Sussex and Dr Alun Jones, deputy editor of *Nature* during 1972-73 and since then with the British Steel Corporation. Professor Roger Williams, of the department of government at the University of Manchester, will also join the centre on secondment from the beginning of 1982.



Jones: from *Nature* to nurture

The centre owes its existence to a substantial grant from the Leverhulme Trust and a consortium of research councils as well as to the now-defunct hope that there should be a centre in Britain equivalent to the Brookings Institution in Washington. Sir Bruce Williams and his deputy, Dr A.J. Kennedy, have been at work since July, and a research fellow started in September. With the appointments now announced, the centre is planning a pause on recruitment while consulting in government and industry on promising lines of enquiry.

One of the research projects at the centre will be the role of incremental changes in technology in the economies of industrial states. Other projects planned include a study of the response of managers to new projects, and in particular of their reactions to various motivations, the importance of new firms in the introduction of innovations and the launching of major manufacturing developments on the basis of research and development.

At least two of the centre's projects will be educational, concerned with the British educational system as an inhibitor of technical change. The centre says, however, that it is not exclusively concerned with change as a harbinger of economic growth but also with criteria for helping to strike a balance "between economic growth and improvements in the environment at work and at large".

Brazilian reactor

Unrestricted uranium

Rio de Janeiro

The Vice-President of the United States, George Bush, announced during his recent official visit to Brazil that the country was free to buy enriched uranium from elsewhere than the United States for the first reloading of its United States-built nuclear reactor Angra I.

The 620-megawatt Westinghouse plant will be fully operational by the end of 1981 and the first reloading will be due in two years' time. The gradual hardening of United States legislation on nuclear safeguards, coupled with the initial contract's demand that refuelling be provided exclusively by the United States, caused enough problems but Brazil's refusal to sign the Non-Proliferation Treaty made it practically impossible for the United States Department of Energy to authorize the exportation of enriched uranium to Brazil.

Following discrete negotiations between the Brazilian utility company Furnas and the United States Department of Energy, Mr Bush announced a diplomatic solution: he has bypassed United States law, declaring that "the Secretary of Energy opened an exception so that the penalty clause of the nuclear contract with Brazil could be dispensed with in the case of Brazil's purchase of the next fuel load for Angra I . . . Brazil will acquire the next fuel load from another source". Brazil is already negotiating the acquisition of fuel rods from Eurenco.

This generosity may be short-lived as Mr Bush later reiterated that Brazil should join the category of "recently industrialized nation" and thus lose the benefits of economic and financial aid reserved for "developing" countries. **Maurice Bazin**

Yugoslav universities

Student mirage

The University of Kosovo in Pristina, the scene of last spring's demonstrations by students from Yugoslavia's Albanian ethnic minority, has mysteriously shrunk by more than 50 per cent during the course of the summer. After the demonstrations, a major investigation and reorganization of the university was carried out by a special party action committee, headed by Dr Redzep Gasi. Every student and academic in the university was screened politically, a process officially described as "differentiation". At the end of the investigation, it was found that the university did not have the 45,000 students it claimed last spring but a mere 20,434.

Last June, the Belgrade authorities had urged that the University of Kosovo should be reduced in size, to correspond with the needs of the Autonomous Province of Kosovo — whose total work force numbers

only some 180,000 — and admissions for the current years were accordingly reduced by 20 per cent. At the end of August, however, it was announced that the university was, in any case, less than half the size that had been supposed.

The whereabouts of the "missing" students are not clear. Part of the discrepancy is probably due to bad book-keeping. During the party purge, it was found that out of the 4,500 supposed members of the university's party organization, more than 1,000 had long since left the university. Since about 10 per cent of the students belong to the party, there are presumably a further 10,000 former students whose names have never been removed from the university's rolls.

Local patriotism seems also to have played some part. The university authorities have been willing enough to boast of rapid growth for an institution founded only ten years ago (with 10,000 students) as a central part of the development of the province.

The main reason for the discrepancy, though, appears to have been financial. As one of Yugoslavia's underdeveloped regions, Kosovo is eligible for federal subsidies which, in the case of the university, are based on the number of students. Commentators have suggested, therefore, that the figures had been deliberately doubled to obtain a bigger grant from Belgrade. **Vera Rich**

Press Council adjudicates

Following a complaint by Dr P. F. Browne of the University of Manchester Institute of Science and Technology that *Nature* breached its announced policy by failing to return an unpublished manuscript, the Press Council has issued the following adjudication:

Nature assures potential contributors that it will return one copy of manuscripts it decides not to publish. The accounts the Press Council has been given of the submission of material by Dr Browne and the exchanges between him and *Nature* have been detailed and complicated: within four weeks at the start of this matter he submitted nine copies of his manuscript in three versions.

His complaint about the failure to return manuscripts relates to those submitted before the present editor assumed the editorship.

The council finds no evidence of a deliberate breach by *Nature* of its announced policy of returning manuscripts but the journal has been unable to produce any evidence to support its belated assertion that the manuscripts were returned to Dr Browne and the council is satisfied he did not receive them.

It is incumbent on a journal of this type to keep a clear and accurate account of the way it has dealt with each manuscript sent to it.

The complaint against *Nature* is upheld.

CORRESPONDENCE

Bradford University

SIR — I write to draw attention to a serious mis-statement in an article on page 248 of *Nature* of 24 September. I do so because its damaging implications have caused grave concern amongst a number of my colleagues who realize how much trust your readers put in the reliability of what is reported in your journal.

The facts are as follows. In September your colleague, Judy Redfearn, explained to me on the telephone that she was writing an article on the reactions of various universities to the contents of the letters they received from the UGC in July last, and she asked me to indicate what was happening at Bradford. I explained that we had initiated a process of consultation and wide discussion which would seek out "grass roots" opinion and culminate in decisions by our senate in December on the new academic profile for the university. She then pressed me to say what we were going to do about the specific advice given by the UGC, such as a possible closure of Biological Sciences. I repeated that the matter was under discussion since the UGC had indicated to universities that an opportunity would be given for the consideration of alternative proposals but that these would have to be contained within the total student numbers allocated to sciences in the university.

It is surprising to find that what was a description of an option open to us becomes not only a statement of a preference (a policy decision) but a selection of which specific areas would thereby be penalized.

I wish to state most emphatically that the University of Bradford has *not* decided that it "would prefer to cut its physics and chemistry departments rather than biology". Besides being untrue the statement of 24 September destroys the internal consistency of your article where it is correctly reported that we shall not be making any decisions until December.

F.M. WILLIS

University of Bradford,
Bradford, West Yorkshire

WE apologize to Professor Willis for having misconstrued his meaning — Editor, *Nature*

Sheldrake's truth

SIR — I read with interest and alarm your editorial "A Book for Burning?" (*Nature* 24 September p.245), in which you criticized Rupert Sheldrake's new book *A New Science of Life* (Blond & Briggs, London, 1981). In particular, a strong, sometimes even hysterical, attack was directed at Sheldrake's alleged belief in the "failure" of molecular biology and at his "vague notion" that the idea of morphogenetic fields, developed by embryologists such as Conrad Waddington and elaborated mathematically by theoreticians such as Rene Thom, can find wider application in the life sciences. Sheldrake's views were denounced as "pseudoscience", "populist" and as introducing "magic" into science. It was implicit in the editorial that Sheldrake is to be considered as an exponent of the intellectually bankrupt nineteenth-century doctrine of vitalism, which has quite rightly been forced to yield place to the subsequently productive (but arguably no less mystical) reductionist

schools of thought.

If I understand Sheldrake correctly, it seems that his conception of molecular biology is not as a sterile failure, but as an important and crucial contribution to the analysis of problems of intracellular organization on which the physiology of the whole organism clearly depends. All Sheldrake appears to have done, then, is to state that the whole is not just the sum of its constituent parts and that higher organizational states cannot be understood in reductionist terms. Although his book is not without its scientific solecisms, Sheldrake has raised many stimulating arguments, and presents an important landmark in the application of a formal geometry to living things as begun by Waddington and Thom. Certainly, I feel that the book is too important to dismiss easily, and in conclusion I should like to recall Milton's dictum that "... Truth never comes into the World but like a Bastard, to the ignominy of him that brought her forth". (*The Doctrine and Discipline of Divorce*, 1643-44).

M.T. ISAAC

St Bartholomew's Hospital Medical College,
London, UK

Appeal to humanity

SIR — With a great feeling of sorrow, we have learned of the latest frequent violations of human rights in the Soviet Union. This time it was the arrest of a physicist, Dr A. Paritsky, together with his wife, living at Str. Tonkopiya 19/2 apt. 48, Kharkov 310091, USSR. Their only fault was a deep desire to live in Israel. In all countries of the free world, any person can leave the country where he was born, but only in the Soviet Union is this considered to be treason. Many Soviet Jews would like to leave the Soviet Union owing to anti-semitism which becomes stronger every day. Among them are many scientists. However, the government of the Soviet Union not only fires them immediately, but in most cases also refuses them the fulfillment of their legal rights. The result of these actions is a vicious circle: on the one hand, some Jewish scientists in the Soviet Union are deprived of the possibility of working according to their speciality, and thus earning their living, but on the other hand they are not allowed to leave the Soviet Union.

An example of this situation is the fate of Dr Paritsky and his family. The family applied for exit visas five years ago. Dr Paritsky and his wife were fired immediately from their work, after that they were refused immigration to Israel, and now they have been arrested.

I would like to apply through your journal to the world scientific community to raise their voices in the defence of the Paritsky family and other Soviet Jewish scientists waiting for exit visas. We must demand that the government of the Soviet Union release Dr Paritsky and give his family permission to emigrate to Israel. I cannot agree with the argument that this action is interference in the internal affairs of the Soviet Union, since the defence of human rights is our common task. On the contrary, we have a bad historical experience in the silence of the international scientific community in the thirties which allowed the Nazi government to do what it wanted.

BORIS S. KRUMGAZ

Israel Oceanographic and
Limnological Research,
Haifa, Israel

Lab. explosion

SIR — A violent explosion occurred recently in our laboratories, following a routine preparation and crystallization of cobalt (II) perchlorate [cobalt (II) chlorate (VII)]. Full details are not yet available, but we understand that between 10 and 20 g of the salt had been prepared by allowing aqueous perchloric acid to react with an excess of cobalt (II) carbonate, filtering off the excess carbonate, reducing the volume and crystallizing the salt, and that the explosion occurred when the caked mass of crystals was placed in a mortar and tapped gently to break it up. No organic matter is thought to have been present, but adventitious material cannot of course be ruled out. The explosion punched a hole through a standard teak bench-top, and fragments of the mortar were ejected through the window glass several metres away.

Pure samples of this compound are normally handled casually without incident, and very little has been found in the literature to indicate any risk of detonation under mild mechanical shock. Cobalt perchlorate and similar salts are readily available commercially, and the bottles carry little or no hazard warning. In the absence of any clear explanation for our explosion, we suggest that it is prudent to handle these materials with extreme caution, and that any form of mechanical disturbance (scrapping, tapping, grinding) of the dried salts should be rigorously avoided.

We hope to report further on the circumstances of the incident following detailed investigations, and would be pleased to hear of any similar accidents or any relevant information.

P.J. ROBINSON

Department of Chemistry,
Manchester Polytechnic, Manchester, UK

Naming names

SIR — Upon my return from vacation, I was taken aback to discover that I was referred to in your editorial of 3 September (p.2) as one of the creators or co-authors of the works of Isadore (Isidore) Nabi. This is completely untrue.

I am shocked that you make this allegation without checking with the people concerned, presumably on the basis of claims by some third party who prefers to hide behind the anonymity of a *Nature* editor. The error in relation to my own role causes me to doubt the accuracy of the rest of the claims in the editorial, including those about Professor Nabi. I have now had the opportunity to read two of the Nabi papers, "On the tendencies of motion" and "An evolutionary interpretation of the English sonnet". Both are satirical works, the former on systems ecology and the latter on sociobiology. In no way are the views of the author hidden or invested with false authority due to the author's name.

I will consider the publication of this letter a sufficient rectification of your error and am willing to terminate my interest in the matter.

RICHARD LESTER

Harvard University,
Boston, Massachusetts, USA

RICHARD LESTER is believed to be a pseudonym of Richard Levins, one of the true culprits — Editor, *Nature*

NEWS AND VIEWS

Calcium and the control of synaptic strength by learning

from E.R. Kandel

In a remarkable convergence of ideas, Ramon y Cajal, the great Spanish anatomist, and Sigmund Freud, the founder of psychoanalysis, independently postulated in 1894 that learning might produce prolonged changes in the effectiveness of the synaptic connections between neurones and that these changes could serve as the mechanism for memory. The idea was not further explored until 1948, when the Polish psychologist Jerzy Konorski¹ put it into the contemporary context of cellular neurophysiology. He suggested that sensory stimuli could produce two types of change in nerve cells and their connections, an invariant and transient *excitability* change, and a facultative but enduring *plastic change*. Konorski postulated that appropriate combinations of stimuli produce the invariant types of excitability changes but also give rise to plastic changes that create permanent functional transformations in a system of neurones.

To determine if synapses undergo plastic changes and to relate these changes to learning it has been necessary to develop suitable cellular techniques and experimental systems. Studies aimed at these problems have involved two inter-related classes of experiments. First, it was necessary to see whether any of the connections between nerve cells showed plastic capabilities; and then to see whether instances of learning actually resulted from particular forms of synaptic plasticity.

During the past two decades there has been significant progress along both lines. A large body of experiments has now affirmed that many chemical synaptic connections can undergo plastic changes. Four classes of synaptic modulation have been described: two types result from changes in activity of the presynaptic neurone of the synapse (homosynaptic plastic change); two others result from activity in related neurones that converge on the synapse (heterosynaptic plastic change). The duration of these plastic

changes can range from minutes to weeks.

In addition, work in the invertebrate *Aplysia* by Kandel, Castellucci and their colleagues has now shown that changes in the strength of specific synaptic connections underlie the memory storage for two simple forms of learning, habituation and sensitization. These changes can last for minutes and hours (short-term memory) or for days and weeks (long-term memory). Finally, recent work in *Aplysia* suggests that different forms of synaptic plasticity may share aspects of a common mechanism — regulation of the concentration of free Ca^{2+} in the presynaptic terminal. For one of these forms of synaptic plasticity, causally related to learning, it has been possible to analyse the molecular events underlying this regulation.

Four types of synaptic plasticity

The experimental study of synaptic plasticity dates from the discovery of the synaptic potential — the physiological link between the activities of the presynaptic and the postsynaptic cell. In 1938 Gopfert and Schaefer², working on the nerve-muscle synapse, discovered this discrete electrical signal interposed between the action potential in the presynaptic terminal of the neurone and the action potential in the muscle fibres. This potential was subsequently shown to result from the ionic currents activated by chemical transmitter released by the presynaptic neurone³. Since 1938, four types of regulatory processes have been described that control the functional strength of chemical synaptic connections. (Other components of the neurone besides synaptic connections also have plastic capabilities but they have been less well analysed and will not be considered here.)

Feng⁴ discovered the first type of synaptic plasticity in 1941 and called it *post-tetanic potentiation*. He found that the synaptic potential at certain synapses can be enhanced for minutes to hours following a brief train of high-frequency (tetanic) activity in the presynaptic neurone. In 1949 Lloyd⁵ discovered a second and reciprocal type of plasticity, *low-frequency depression*, whereby a period of low activity produces a depression in synaptic effectiveness.

Both of these forms are homosynaptic. About 10 years later, Frank and Fuortes⁶ working on the spinal cord and Dudel and Kuffler⁷ working on crayfish neuromuscular synapses provided the first account of heterosynaptic plasticity. Their discovery of heterosynaptic inhibition documented that activity in one pathway can depress the synaptic potential produced in another pathway. (For example, stimulating the inhibitory axon to the muscle of the lobster decreases the amplitude of the synaptic potential produced by the excitatory motor axon to that muscle.) In 1964, Kandel and Tauc⁸ described heterosynaptic facilitation in *Aplysia*, whereby activity in one pathway enhances activity in another.

The finding that there are four ways in which activity can regulate synaptic strength raised two further questions: (1) Which, if any, of these forms of synaptic plasticity are actually involved in learning, and (2) what specific mechanisms account for the changes in synaptic strength?

Synaptic plasticity and simple forms of learning

Analysis of simple forms of learning in the invertebrates *Aplysia* and crayfish indicates that changes in synaptic strength account for the memory storage for such learning. *Habituation*, learning to ignore a stimulus that is repeatedly presented, involves a dramatic form of homosynaptic (low-frequency) depression^{9,12}. In contrast, *sensitization*, learning to attend to a variety of otherwise neutral stimuli as a result of recent presentation of a noxious stimulus, involves presynaptic facilitation^{9,12}. What role in learning, if any, is served by the two other forms of synaptic plasticity, post-tetanic (homosynaptic) potentiation and presynaptic (heterosynaptic) inhibition, is not known. In the crayfish tail-flip response there is evidence that presynaptic inhibition functions to protect the animal from habituation that might be produced by tactile stimuli initiated by its own tail movements.

Synaptic plasticity and free Ca^{2+} concentration

A key to the detailed analysis of each of the

Eric R. Kandel is in the Division of Neurobiology and Behaviour, College of Physicians and Surgeons of Columbia University, New York.

Calcium ion accumulation and current modulation

<i>Ca²⁺ accumulation</i>				
	Intrinsic		Synaptic	
(1) Post-tetanic potentiation	+		-	
<i>Ca²⁺ current modulation</i>				
	Direct modulation (Ca ²⁺ channel)	Indirect modulation (K ⁺ channel)	Intrinsic	Synaptic
(2) Homosynaptic depression (habituation)	+	-	+	-
(3) Presynaptic inhibition	+	-	-	+
(4) Presynaptic facilitation (sensitization)	-	+	-	+

four types of plasticity was provided when it became possible to specify whether the mechanism of the plastic changes was presynaptic (involving a change in transmitter release) or postsynaptic (involving a change in receptor sensitivity). In 1954, del Castillo and Katz⁵⁰ demonstrated that chemical transmitter is released from the presynaptic terminal in discrete multimolecular packets, or quanta, each containing several thousand molecules. With this finding came the ability to analyse the relative contribution to synaptic transmission of changes in pre- and postsynaptic elements. When a quantal analysis was applied to each of the four types of plasticity^{7,12,13,14} the locus of plasticity was shown in each case to be presynaptic: the number of transmitter quanta released per impulse was altered but the sensitivity of the receptor did not change.

In 1967 Katz and Miledi¹⁵ found that the release of transmitter quanta depends on the influx of Ca^{2+} that occurs with each action potential. They proposed that Ca^{2+} facilitates the binding of synaptic vesicles to release sites. Binding of these transmitter-filled vesicles is a prerequisite step for the exocytosis of transmitter. On the basis of these findings Katz and Miledi¹⁶ and subsequently Rahamimoff^{17,18} suggested that changes in the availability of intracellular free Ca^{2+} might be important for certain forms of short-term synaptic plasticity. Rahamimoff proposed that the intracellular concentration of Ca^{2+} might be regulated by the organelles that buffer Ca^{2+} in the presynaptic terminal — the mitochondria and endoplasmic reticulum. In support of this idea, Rahamimoff found that post-tetanic potentiation seemed to result from an accumulation of Ca^{2+} inside the presynaptic terminals because of Ca^{2+} entry during each action potential of the tetanus and, perhaps additionally, because of its subsequent release from internal stores by Na^{+} . This residual Ca^{2+}

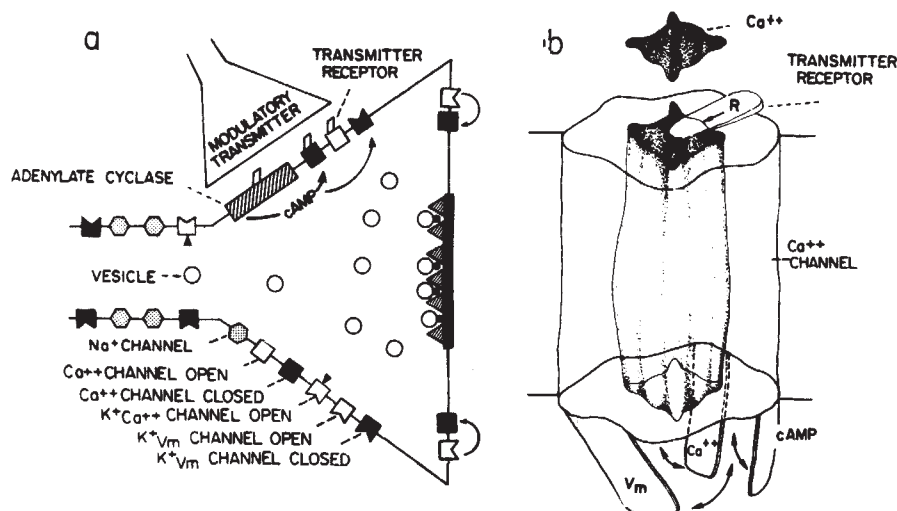
accumulates in the terminal in the aftermath of a series of action potentials and is taken up only slowly by the buffering organelles.

A direct test of the residual Ca^{2+} hypothesis requires that the presynaptic terminal be impaled for intracellular recording, something which has not yet been possible at the nerve-muscle synapse. In addition, these indirect analyses of the post-tetanic potentiation have been compromised because tetanic stimulation also gives rise to changes in membrane potential in the presynaptic terminals that may have secondary effects on release, masking the actual time course of the true facilitation.

Some of these problems have now been overcome in the large neurones of *Aplysia*. The membrane of the cell body of *Aplysia* neurones contains Ca^{2+} channels whose properties resemble those of the membrane of the presynaptic terminal¹⁹⁻²². In certain conditions changes in the Ca^{2+} current of the cell body parallel the changes in transmitter release at the terminals²³. Moreover, in some of these neurones the presynaptic terminals controlling transmitter release are sufficiently close to the cell body electrically so that it is possible to modify transmitter release from the terminals by injecting current into the cell body²⁴⁻²⁶. These observations suggested to Klein, Shapiro, Kretz and Kandel that it might be possible to examine the relationships between transmitter release on the one hand and Ca^{2+} accumulation or changes in Ca^{2+} influx on the other. They combined analysis of ionic currents of the cell body of the presynaptic neurone, using voltage-clamp analysis and pharmacological blockade of specific ion channels, with assay of transmitter release from the presynaptic cell, using intracellular recordings of the synaptic potential in the postsynaptic cell. Klein, Kretz, Shapiro and Kandel have applied these techniques to two identified synaptic connections in the abdominal ganglion of *Aplysia*: connections made by the cholinergic cell L10, and connections between the sensory neurones and the motor neurones of the gill-withdrawal reflex.

Kretz, Shapiro and Kandel²⁷ examined post-tetanic potentiation and found that the enhancement of synaptic strength is

Fig. 1 Models of modulation of Ca^{2+} current. (a) Schematic model of synaptic terminal release area, illustrating several intrinsic membrane proteins, the voltage-dependent Na^{+} channel, voltage- and Ca^{2+} -dependent K^{+} channels, and Ca^{2+} channels. Channels can be opened or closed. Ca^{2+} current can be modulated indirectly by increases or decreases in K^{+} current. Ca^{2+} and K^{+} channels can also be modulated by neurotransmitter action. At least three processes at the terminal may be dependent on intracellular Ca^{2+} concentration: (1) transmitter release, (2) Ca^{2+} -dependent K^{+} activation, and (3) Ca^{2+} channel inactivation. Cyclic nucleotides may also regulate membrane K^{+} and Ca^{2+} conductances. v_m , membrane potential. (b) Schematic model of a Ca^{2+} ion channel. Membrane conductance may be modulated by four regulatory signals: (1) membrane voltage, (2) intracellular Ca^{2+} concentration, (3) second messengers, and (4) modulatory transmitters (from reference 30).



paralleled by a Ca^{2+} -activated K^{+} current in the presynaptic cell L10. This K^{+} current is activated by an increase in intracellular Ca^{2+} concentration, and there is good evidence that it provides an index of the amount and time course of the increase in Ca^{2+} . To test further the idea that post-tetanic potentiation results from an increase in intracellular Ca^{2+} , the accumulation of Ca^{2+} during potentiation was blocked by injecting the Ca^{2+} chelating agent EGTA into the presynaptic neurone²⁸. As predicted by the residual Ca^{2+} model, the injected EGTA decreased the Ca^{2+} -dependent K^{+} current, the size of postynaptic potentials, and the duration of the post-tetanic potentiation.

Plasticity and Ca^{2+} influx

Using the same approach, homosynaptic depression, presynaptic inhibition and facilitation were also examined²⁹. Here a novel mechanism — modulation of the Ca^{2+} influx into the presynaptic terminal was encountered. It was found that the influx of Ca^{2+} with each action potential is not constant — indeed, the action potential in the terminal is not all-or-none. Rather, the contribution of Ca^{2+} influx to the action potential can be regulated in both directions and this regulation can be direct or indirect. *Direct modulation* of the Ca^{2+} current is involved in homosynaptic depression (the mechanism of habituation), and presynaptic inhibition^{29,30} and is achieved either by a direct action on the Ca^{2+} -channel protein itself or by action on a regulatory protein intimately associated with the Ca^{2+} channel. (Mudge *et al.*³¹ have reached a similar conclusion based on their analysis

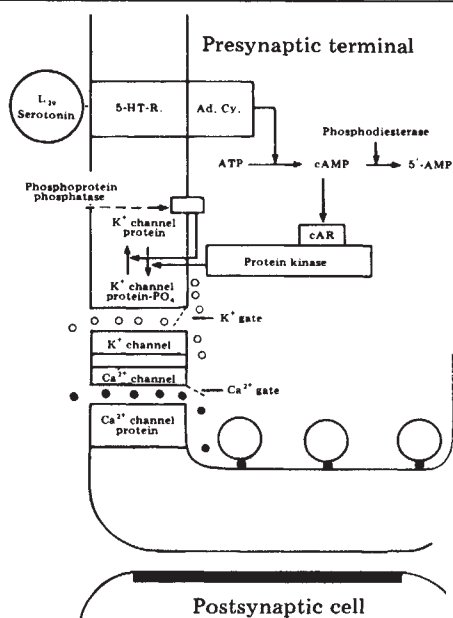


Fig 2 A molecular model for presynaptic facilitation (from reference 32).

of presynaptic inhibition in dorsal root ganglion cells in dissociated cell culture.) *Indirect modulation* of the Ca^{2+} current is involved in presynaptic facilitation^{23,32} (the mechanism of sensitization). It is achieved through regulation of the opposing K^{+} current that controls the height and duration of the action potential and therefore determines the time during which the Ca^{2+} channels are activated (see the table).

Ion channels are thought to be intrinsic membrane proteins. These experiments

suggest that the ability to modulate the Ca^{2+} current directly and indirectly derives from an interesting characteristic of the Ca^{2+} and the K^{+} channel proteins in the terminals: the existence on these channel proteins of several regulatory sites (see figure one). On the basis of the data in *Aplysia*, obtained by Klein, Shapiro and Kandel, and those of others^{31,33-36}, at least four regulatory sites can be identified on the Ca^{2+} and the K^{+} channels. These sites are sensitive to the following regulatory signals: (1) membrane voltage, (2) chemical transmitters, (3) cyclic AMP and (4) intracellular Ca^{2+} concentration. Thus the existence of several regulatory sites on the Ca^{2+} channel (or on other proteins that interact with the channel) and the additional control achieved through actions on the K^{+} channel and by Ca^{2+} accumulation provide a mechanistic explanation for each of the four different known forms of short-term plasticity. Although these forms of short-term plasticity vary in their detailed mechanisms, they all share a common mode of expression — the modulation of Ca^{2+} concentration.

Toward a molecular explanation of synaptic plasticity

The voltage clamp analyses outlined above allow the mechanisms underlying homosynaptic depression and presynaptic facilitation and inhibition to be attributed to changes at particular ionic channels. As membrane channels are thought to be integral membrane proteins the ability to specify particular channels for presynaptic facilitation and inhibition is an essential first step towards a biochemical analysis of their underlying mechanisms.

In the case of presynaptic facilitation available information allows a specific, but tentative, molecular model to be considered. The data also provide the first direct evidence that cyclic AMP-mediated phosphorylation can control synaptic strength. Kandel, Klein and their colleagues have found that serotonin is the likely transmitter for presynaptic facilitation. Serotonin has been found to stimulate the synthesis of cyclic AMP in the synaptic terminals, and cyclic AMP can in turn enhance transmitter release by depressing the K^+ current and thereby increasing Ca^{2+} current. Direct intracellular injection of the catalytic unit of the cyclic AMP-dependent protein kinase indicates that the serotonin-stimulated increase in cyclic AMP enhances the activity of a cyclic AMP-dependent protein kinase in the presynaptic terminals of the sensory neurones and that the kinase phosphorylates and thereby closes the K^+ channel³⁷ (see figure 2). Intracellularly injecting the specific inhibitor of the cyclic AMP-dependent protein kinase (Walsh inhibitor) blocks the action of serotonin^{37a}. Moreover, L. Bernier, J. Schwartz and their colleagues have found that serotonin and cyclic AMP lead to the phosphorylation of a membrane protein

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with a molecular weight of 160,000 from sensory neurones incubated with ^{32}P . Although it has been known since 1962 that the brain contains one of the highest concentrations of cyclic AMP of all body tissues³⁸, it has been difficult in the past to relate cyclic AMP-mediated protein phosphorylation to any specific neuronal function^{39,39a} because of the small size of nerve cells in the mammalian brain and the inaccessibility of most central synapses. As these studies illustrate, these problems have been largely circumvented by using *Aplysia* and other higher invertebrates: cyclic AMP and protein phosphorylation can now be directly related to the prolonged enhancement of synaptic transmission that underlies a short-term form of memory. Combined biophysical and biochemical experiments suggest that the substrate protein from the cyclic AMP-dependent protein kinase may be either part of a K^+ channel itself or a regulatory molecule. This regulatory protein resembles certain metabolic enzymes whose activities can be modified both by allosteric ligand interactions and by covalent modifications.

Multiple controls of ionic channels

These studies provide new insights into gating mechanisms for ionic channels. It had been known since Hodgkin and Huxley's classic work in the early 1950s that there are two classes of channels in excitable membranes: nongated or leakage channels that are always open, and gated channels that are either closed or opened by membrane voltage. Fatt and Katz added a second subclass of gated channels when they found that the channels activated by acetylcholine at the neuromuscular junction are chemically gated; they are normally closed until acted on by the transmitter. Moreover, Fatt and Katz found that these chemically gated channels cannot be activated by voltage.

In 1957 Grundfest extended this idea by suggesting that all gated channels are only singly gated: they can be activated by either voltage or chemical transmitter, not by both⁴⁰. This insight gave rise to what appeared to be a fundamental distinction in the channels of excitable membranes: voltage gated channels are electrically excitable (but chemically inexcitable) and chemically gated channels are electrically inexcitable. This theory has implicitly guided aspects of research on excitable membranes for 20 years⁴¹ and has been supported by many examples — with only minor exceptions such as the slight dependence on the synaptic current at the neuromuscular junction on membrane voltage⁴²⁻⁴⁴.

However, in the last few years it has become clear that the theory of single gating explains only some gating processes. There exists in addition another category of channels whose gating is doubly or even multiply regulated. These multiply regulated channels were first found in the heart by Reuter³⁵ and Tsien⁴⁵ and in nerve

cells by Kuba and Koketsu⁴⁶, Wilson and Wachtel⁴⁷ and Brown and Adams⁴⁸. The work on *Aplysia* illustrates two novel features of doubly regulated channels. One is that they can occur in the synaptic terminals and participate in control of transmitter release. A second feature is that the multiple gating property of these channels endows the terminals with unusual plastic capabilities because it makes the release of transmitter responsive to electrical as well as chemical modulatory signals from intrinsic or extrinsic sources, or both.

The behavioural and synaptic actions that have been studied under voltage clamp last up to an hour. As indicated above, however, simple preparations also manifest behavioural and synaptic actions that are modulated for periods of weeks. It

will therefore be interesting to extend the approaches outlined here to longer-lasting and ultimately even to more complex learning processes such as classical conditioning. Classical conditioning resembles sensitization in several of its features and differs only in its requirement of temporal specificity in the pairing of stimuli. Kandel and Schwartz⁴⁹ have therefore proposed that conditioning and sensitization share a common memory mechanism — presynaptic facilitation — and that classical conditioning has, in addition, a separate mechanism responsible for the temporal specificity. If this were so, it would indicate that the same molecular regulatory mechanisms for synaptic plasticity could be used for building blocks for different forms of learning. □

Radiocarbon dating and archaeology

from Robert Hedges

THE desirability of integrating the strategic aims of archaeologists with the technical possibilities of modern radiocarbon laboratories was the main motive for a recent meeting at Groningen.* The radiocarbon laboratory there was one of the first ever to be set up and still plays a leading part in radiocarbon studies, and, as might be expected, participation from radiocarbon labs world-wide was strong. The attendance of archaeologists was, by contrast, somewhat disappointing. Acceptance of radiocarbon dates, although once highly controversial, is now quite general but even so material is often submitted for dating in the spirit of adding a scientific precision to the archaeologists' pre-existing beliefs. Cooperation between physical dating and archaeological methods can be much more effective than this, and several examples of such work were presented by those archaeologists who did attend. No doubt in time the advantages of their approach will percolate to their less enlightened bretheren.

The meeting was not for the faint-hearted, however. Much of the time was devoted to examination of the influences that can render a ^{14}C measurement inaccurate or unreliable. A team from the University of Glasgow compared dates of tree-ring groups from twenty laboratories and showed that, with few exceptions, it is difficult to assess individual laboratory errors. Calibration of the radiocarbon time-scale (or, equivalently, measurement of past atmospheric levels of ^{14}C), over the last two millennia were reviewed by Stuiver (University of Washington). In dating tree rings the precision of his laboratory, and that of Groningen and Heidelberg,

approaches the limit set by the faithfulness of the record in individual trees. This record can, however, produce ambiguous calendar dates from a single radiocarbon date. The complexities of the radiocarbon fluctuations in the past can also be put to useful account through 'wiggles matching' that is, matching the pattern of variation in different samples. Becker (University of Hohenheim) and Suess (University of California, San Diego) have used the technique to match a floating section of the European oak chronology to the bristlecone pine chronology and to extend dendrochronological calibration back into the 7th millennium BC.

Tree rings provide a rather idealised type of sample for dating and much discussion was devoted to the problems of dating materials more typical of archaeological sites. Charcoal and bone are by far the most common and both are subject to various forms of contamination. Opinion varied as to which could give the more reliable results, the only 'control' on the reliability of an isolated sample being the degree of satisfaction expressed by the archaeologist as to its radiocarbon date! There are special problems with charcoal as not only can timbers be re-used, but, especially in cold climates, dead wood and drift wood can be preserved for a long time before eventual use in fires. While proper pre-treatment chemistry should remove contamination by soil organics and organisms in charcoal and bone, this is more difficult for carbonates. Robinson (USGS, Menlo Park) presented evidence for a simple contamination model of shell carbonates which severely affects dates older than 15,000–20,000 years. Younger

*The First International Symposium on ^{14}C and Archaeology was held at Groningen, August 24–28, 1981.

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shells should be fairly reliable provided no major aragonite-calcite transformation is detected after routine etching of the surface layers.

In addition to contamination during deposition, many materials sample a ^{14}C content significantly different from that in the atmosphere. Terrestrial snail shells, for example, appear to contain carbon up to a thousand years older than the vegetation they feed on (Evin, Université Claude-Bernard de Lyon). This is analogous to the 'hard water' effect of aquatic plants whose ^{14}C level contains a component from the (^{14}C free) geological carbonate. A similar effect was noted for shells growing in waters subjected to injection of volcanic carbon dioxide. The major reservoir of ^{14}C , the deep ocean, contains significantly older CO_2 , so that marine organisms show an anomalous age, about 300–500 years too large in most coastal waters. The older ^{14}C component can be followed up through the food chain and human populations whose main source of protein is from the sea should also have an older date for their bone collagen. This is actually the case, and can be confirmed by measurements of the stable isotope ratio, $\delta^{13}\text{C}$, (Tauber, National Museum, Copenhagen; Nelson, Simon Fraser University). The ratio reflects an extra isotopic fractionation step attributable to the metabolism of carbon dissolved in the sea rather than the atmosphere. The potential age correction by 400 years or so for a predominantly sea-food eating population, as well as the possibility for identifying ancient economies, could have considerable archaeological significance.

Radiocarbon dates may soon be available from very much smaller samples (down to about one milligram of carbon) with the Zurich accelerator system and the Oxford accelerator/mass spectrometer. The new accelerator methods, with their very high signal to background characteristics, are likely to have their greatest effect on the dating of the Upper Palaeolithic, where samples are scarce and contaminated and many dates are beyond the range of counting laboratories. Technical advances in small counters can, however, now make possible the dating of 'young' samples in the 10–50 mg range (AERE, Harwell).

It is probably these advances in technique which will make the greatest impact on the future collaboration of archaeology and laboratory radiocarbon dating. Gowlett (University of Oxford) outlined the kinds of archaeological programme implied by the new methods, and this was followed by a review by Hours (CNRS, Lyon) of the role of dating in new directions in prehistory which might have been written with accelerator dating explicitly in mind. In any case, there is a good prospect that at the next meeting there will be more material, rather than less, to sustain a dialogue between radiocarbon laboratory and archaeologist. □

Complexity in human histocompatibility loci

from Andrew McMichael and William Makgoba

THE Ia antigens of the mouse are polymorphic cell surface glycoproteins largely restricted to expression on cells of the immune system. They are a product of the mouse histocompatibility complex (H-2) and are crucial for many normal T lymphocyte functions. They are involved in activation of T cells, which can be measured in the mixed lymphocyte reaction (MLR) assay and also in helper and suppressor T cell function. The human equivalent are the HLA-DR antigens which with their clear clinical importance, are currently the subject of intense investigation. Although there seem to be many structural similarities between the mouse Ia and the human HLA-DR antigens and the position of the loci within the histocompatibility antigens and the position of the loci within the histocompatibility complex, Shaw *et al.* and Kavathas *et al.* in this issue of *Nature*, describe the mapping of a gene locus that controls secondary MLR stimulating determinants and is located outside the conventional human histocompatibility complex (HLA).

The nomenclature of the Ia antigens is currently in some dispute but Klein *et al.*¹ have argued forcibly that only the biochemically definable two chain I-A and I-E antigens should be ascribed gene loci. The H-2 I region would comprise only four loci, A_β , A_α , E_β and E_α , controlling the β (27–30,000 dalton) and α (30–33,000 dalton) chains of these molecules. In the mouse, most of the genetic polymorphism lies in the A_β chain of I-A. In addition to the α and β chains there is a polypeptide of about 30,000 daltons, I, which is associated with the ^{35}S -methionine internally labelled Ia antigens and identified by two dimensional gel electrophoresis as a basic spot of invariant position². It is biochemically unrelated to the α and β molecules³.

By analysis of the MLR responses of human T cells, eleven alleles (DW1–DW11) were described at the locus^{4,5} which has been shown by Yunis and Amos⁶ to control the MLR response.

Subsequently, alloantisera were identified which reacted with HLA antigens present on B cells but not (resting) T cells and an allelic series, DR1–DR10 was defined⁵. Correlation between these D related (DR) antigens with the MLR defined (D) antigens was remarkably good and the few discrepancies were generally

attributed to differences between T cell and B cell recognition of antigen.

Immunoprecipitation of HLA DR antigens, using alloantisera and partial sequencing revealed that they were homologous with the I-E antigens of the mouse⁷. While the sera that identified HLA DR antigens were probably typing the products of one locus, some sera were found that identified crossreactive, or supertypic, specificities^{8–11}. Immunoprecipitation studies indicated that at least one of these, DC1, was present on a distinct molecular species⁸. In a highly polymorphic system, however, each molecule might have more than one polymorphic antigenic determinant so that some supertypic specificities could be epitopes shared by two or three HLA DR molecules.

The advent of monoclonal antibodies, two-dimensional gel techniques and antigen specific T cell lines have provided new tools with which to dissect the HLA DR system. Most of the anti DR monoclonal antibodies have recognised monomorphic (that is non-polymorphic) determinants. These reagents precipitate the α (30–33,000 dalton) and β (27–30,000 dalton) chains from cell membranes. There is evidence that some of these antibodies distinguish two sets of HLA DR antigens. Lampson and Levy¹² and Nadler *et al.*¹³ have described monomorphic antibodies which fail to clear all the Ia-like antigen in immunoprecipitates. Accolla *et al.*¹⁴ have found two monoclonal antibodies that precipitate DR antigens with different α and β chains, defined by peptide mapping.

Two-dimensional gel techniques were first applied with monoclonal antibodies specific for monomorphic epitopes, when a complex pattern of spots was revealed¹⁵. Part of the heterogeneity would be removed by preincubation of the cells, before membrane labelling, with tunicamycin to block glycosylation, but at least five spots remained. One of these was the invariant, I, spot, equivalent of that in the mouse; the others comprised α and β chains. Comparison of the spot pattern derived from HLA DR homozygous cells showed that the β spots were variable and the α constant. De Kretzer *et al.*¹⁶ have recently compared two-dimensional gel patterns prepared with monomorphic antibodies from homozygous cell lines derived from donors who were offspring of consanguineous marriages. They found examples of homozygous cells of the same HLA DR type with different β spot patterns; they suggested that the β spots could be divided into two groups, one identical in DR matched cells and the

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other, presumably identifying products of a different locus, which was variable.

Alloantisera specific for HLA DR3 and the supertypic specificities MT2 and MB2 were used by Markert and Cresswell¹⁷ to precipitate antigen from an HLA DR3 homozygous B cell line. Two-dimensional gel analysis revealed distinct α and β chains for the MT2 antigen and possible further heterogeneity in the α chain associated with MB2. Shackleford *et al.*¹⁸ have studied HLA DR2 and 6 and the associated supertypic specificity DC1 (also known as MT1 and MB1) with specific alloantisera and a monoclonal antibody, Genox 353, specific for DC1. It was found that the α and β chains of DC1 were of slightly lower molecular weight than those of DR2 and DR6. On two-dimensional gels the DR and DC spot patterns were distinct and there was evidence that the DC1 β spots associated with DR2 were different from those found with the DR6 haplotype. Corte *et al.*¹⁹ prepared peptide maps from DC1, purified with a DC1 specific monoclonal antibody, and found differences between DC1 and DR2, but no evidence for polymorphism in DC1. It is interesting that DC1 is identified by a monoclonal antibody, MRC OX3, which reacts with an antigenic specificity (Ia9) associated with the I-A molecule of H-2 (ref. 20).

In the light of the many structural similarities between H21a and HLA-DR antigens the reports in this issue of *Nature* by Shaw *et al.* (page 745) and Kavathas *et al.* (page 747) describing the mapping of a gene locus that controls secondary mixed lymphocyte reaction stimulating determinants, SB, are very interesting. These polymorphic SB antigens, with five alleles defined so far, stimulate proliferation of T cell lines that have been grown *in vitro* by repeated challenge with cells of a particular SB type (but matched for HLA DR). Each line is entirely specific for one SB

determinant. In a series of families in which HLA recombination had occurred, the SB locus was mapped between HLA DR and the glyoxylase locus, GLO. In a study of mutant cell lines which had ceased to express the HLA DR antigens, one of which showed a deletion on the short arm of chromosome 6, the SB stimulating locus was retained. This study therefore maps SB well outside the conventional boundaries of HLA. It is not clear at this stage whether SB is the equivalent of a known H-2 I region product and it will be interesting to see which monoclonal antibodies, if any, inhibit recognition.

To summarize, the data indicate that the HLA DR region is indeed complex. There appear to be several loci with products definable as cell surface antigens. A minimum hypothesis would have to include at least four loci, coding for the α and β chains of HLA DR and DC, which are probably equivalent to I-E and I-A respectively. If SB is Ia-like, this would constitute a third series with no apparent homologue in the mouse so far defined.

The complexity may be much greater. Shackleford *et al.*¹⁸ have indicated that hybrid molecules may exist between the two types of α and β chains that they have identified. Also there is the possibility that some of the DR specificities might be carbohydrate epitopes which may also be polymorphic²¹. Future studies, particularly peptide mapping of antigens purified by monoclonal antibodies and direct identification of the HLA DR genes by DNA probes, should clarify the situation.

The importance of HLA DR lies in its role in T cell activation. This is clinically relevant in transplantation where HLA DR matching is probably more important than HLA ABC matching²². Many human diseases are associated with particular HLA DR antigens²³, for instance multiple sclerosis with HLA DR2, organ-specific autoimmune diseases with HLA DR3 and rheumatoid arthritis with HLA DR4. A better understanding of the HLA DR complex might reveal the underlying reasons for these relations.

Mechanisms of cell mediated lysis

from Elizabeth Simpson

THE MECHANISM by which lymphoreticular cells kill target cells is much less well understood than target cell lysis by antibody and complement. A recent international workshop* was designed to explore what was known of mechanisms of cell mediated lysis and to make comparisons between the different types of cell mediated killing and to relate them, where possible, to complement mediated kill.

Lysis by T cells both specific (TCTL) and lectin mediated (LDCC), by K cells plus antibody (ADCC), by natural killer (NK) cells and by macrophages were considered. Recognition and binding of the target cell precede the 'lethal hit' and the subsequent disintegration of the target cell. The recognition step can be separated from the lethal hit by the dependence on the presence of calcium in the medium of the lethal hit. Recognition and binding without the killing step can take place in the absence of calcium ions.

Time lapse microcinematography (Sanderson, NIMR, London; Zagury, Université Pierre et Marie Curie, Paris) provided evidence that there is a long and variable time (15 min – 3 hours) between T and K effector cell attachment to a target, and lysis, although the final lytic step is rapid and involves violent movement of both attacker and target. The attacking cell

then detaches and can move on to another target cell. The tempo of this reaction is certainly very different from that produced by antibody plus the complement cascade, which results within a very short time in the target cell membrane being plugged with a membrane attack complex which renders it leaky. (Lachman, MRC, Cambridge; Mayer, Johns Hopkins School of Medicine).

The mechanism of kill has been analysed by several people working with cloned cytotoxic T cell lines and/or monoclonal antibodies directed against cell surface determinants on effector cells (Fitch, University of Chicago; Glasebrook, Swiss Institute for Experimental Cancer Research; MacDonald, Ludwig Institute; Martz, University of Massachusetts; Schmitt-Verhulst, Golstein, Pierres and Malissen, Centre d'Immunologie de Marseille-Luminy). T cytotoxic lymphocytes (TCTL) bind to their targets by specific receptors which recognize glycoproteins of two different sorts, each coded by the major histocompatibility complex (MHC), H-2 in mouse, HLA in man (see Klein *et al.* *Nature* 291, 455). The first type, called class I, is exemplified by the H-2K and D molecules in mouse, and HL - A, B and C molecules in man, and determinants on these molecules have been

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*The first international workshop on mechanisms of cell mediated lysis was held in Marseille, September 13-17, 1981 and was organized by W.R. Clark and P. Golstein.

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defined serologically using alloantibodies. The second type, called class II, are the H-21 region associated antigens of mouse, and the HL DR determinants of man. These can now be identified with appropriate alloantibodies, but were initially discovered by their capacity to stimulate strong proliferative responses in mixed lymphocyte culture (MLC).

The activity of cloned cytotoxic lines directed against class I major histocompatibility (MHC) antigens can be blocked by monoclonal antibodies against Ly2. This affects the recognition step only, and is reversible. In contrast, cytotoxic clones directed against class II MHC antigens are not blocked by anti Ly2. Monoclonal antibodies directed against neither Ly1, nor MHC class I or II molecules on the effector cell block cytotoxic T cell function. A very interesting group of monoclonal antibodies directed against a cell surface glycoprotein (molecular weight $\sim 180,000$) on cytotoxic T cells were reported by several laboratories. In each case, the antibody was raised against cytotoxic T cells, and blocked cytotoxicity of T cell clones against class I or II MHC antigens. However, the role of the molecule(s) in lysis is not clear, and may not even be essential, since cloned cytotoxic lines lacking the antigen are reported. In the case of some of these cytotoxicity blocking monoclonal antibodies, NK cytotoxicity could also be partially blocked. A specific cytotoxicity blocking monoclonal antibody was reported by Fitch, against his L3 cytotoxic clone. This could be an antireceptor antibody.

Another analytic approach using somatic cell hybridization has been taken by other groups. Cytotoxic T cell clones (Nabholtz, Swiss Institute for Experimental Cancer Research) or trypsin treated bulk mixed lymphocyte cultures (Kaufmann, Weizmann Institute) were fused with a HAT sensitive T cell tumour, BW5147 and hybrids were grown in the presence or absence of T cell growth factor. Nabholtz stressed the importance of using karyotypically normal, stable cytotoxic clones isolated using concanavalin A free TCGF, filler cells and antigen for such fusions and obtained from them functionally active (cytotoxic), but TCGF dependent hybrids. From them, however, TCGF independent variants would occasionally arise spontaneously, although their functional activity was more variable. This was comparable with data shown by Glasebrook that cytotoxic T cell clones independent of externally added TCGF occurred, but these clones produced TCGF following activation by specific antigen. The cytotoxic hybrids reported by Kaufmann were independent of TCGF but no production of TCGF by them had been detected. They had retained functional activity after many months *in vitro* and could also be grown *in vivo* and retain activity. One of the aims of those producing cytotoxic hybrids is to produce genetic

mutants which can then be analysed functionally and genetically, for example, by cell surface markers, to determine correlations between structure and function and so approach more directly the question of mechanism of T cell kill. Fitch approached the problem by mutagenizing not hybrids but cytotoxic clones, and then selecting with various monoclonal antibodies plus complement. Clones thus selected by anti Ly2 plus complement were Ly2 negative and had lost specific cytotoxic function, although they could still kill nonspecifically in the presence of the lectin concanavalin A.

Killing of target cells by macrophages seemed at least in some instances to be a much slower process than T cell killing, and there was evidence that some tumour targets could resist macrophage attack by effecting repairs (Hanna, Frederick Cancer Research Center, Maryland). Nathan (Rockefeller University) reported that macrophages secrete many substances, including arginase, proteases and hydrogen peroxide and that there were several pathways by which macrophages could kill, tumour cells for example, some of which require high levels of arginine.

NK cell killing is not easy to study because it is variable, levels of activity cannot be raised by immunization and there is an incomplete understanding of

what target cells determinants are recognized. Certain tumour lines are very sensitive to NK killing and others are not. In mice, there is a remarkably good correlation between failure of a tumour to grow *in vivo* and sensitivity to NK cytotoxicity *in vivo* (Keissling, Karolinska Institute; Henney, Fred Hutchinson Cancer Research Center, Seattle). NK cell activity in mice is rather low (in comparison with man) and is missing in some mutant strains (the Beige mutant). In man, cells enriched for NK activity have been isolated from peripheral blood and identified as large granular lymphocytes (Herberman, NIH). Electron micrography shows them to contain granules and polymerized tubules which may be excreted during lysis of target cells (Henkart, NIH).

Target cell destruction by lymphoid cells is probably different from that of antibody plus complement, although the production of a membrane attack complex plug by a process analogous to that of the complement cascade remains an attractive hypothesis to some (Lachmann and Meyer). Use of a combination of cytotoxic T cell clones and monoclonal antibodies provides a powerful approach that may be expected to yield information as to the identity of the various components of the killing mechanism, including even perhaps that continuing enigma, the T cell receptor! □

Problems with solar oscillations

from Douglas Gough

THE increasing precision with which solar oscillations have been measured in recent years has enabled us to make interesting inferences about the structure of the Sun. As is well known, previously available data cannot all be incorporated into the theory. In particular, only if the abundances Y and Z of helium and heavy elements are presumed to be well below the values that are generally believed, will the neutrino luminosity be low enough to agree with observation. Independent measures are thus of great importance.

To date, only oscillations with periods near five minutes have provided useful diagnostic information. The modes of high degree, first measured with adequate precision by Deubner¹, have taught us that the convective envelope is at least 200,000 km deep. Within the confines of standard theory, this could be so only if $Y < 0.25$ and $Z < 0.02$, which is indeed in accord with most astronomers' beliefs. More recently, similar abundances have been inferred from oscillation modes of low degree. Thus, on the whole, the different kinds of oscillation are pointing to a similar

theoretical model, although that model would produce more neutrinos than are actually observed. That may be telling us something about the weak interactions in which the neutrinos are created, or perhaps it is providing evidence that neutrinos have mass.

In principle, oscillation frequencies can give us much more information about not only the chemical composition, but also the density and temperature stratification and the distribution of angular momentum. The amount of data is increasing rapidly, and there is reason to be hopeful that accurate measurements of some aspects of the Sun's interior structure will be possible in the foreseeable future. At the recent IAU Colloquium 66 these data were compared and discussed. It was learnt that there remain several questions to be answered before they can be put to good use.

Diameter measurements

An analysis of the temporal power spectrum of solar diameter variations between 400 and 430 μHz showed peaks at 416.2, 414.1 and 420.7 μHz (H.A. Hill, University of Arizona). There are only a few oscillation eigenfrequencies of the Sun in this range, and one is not hampered by interference between unresolved modes. The peaks are listed in order of an

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increasing width which measures, Hill suggested, the separation of unresolved rotationally split components. The oscillations are probably p modes of low order and the three modes can be tentatively assigned degrees of 0, 2 and 3 or 4 respectively. The analysis will no doubt provide a valuable diagnostic for the solar interior.

Five-minute oscillations of high degree

The latest measurements from the Kitt Peak Observatory (E.J. Rhodes, Jr, University of Southern California) showed phase coherence of the motion to be maintained over two consecutive days. There is thus hope that the accuracy with which the dispersion relation is measured can be improved, so increasing the diagnostic power of the observations. Rhodes also reported that the apparent variation of rotational splitting reported earlier² could no longer be detected, and was probably a result of instrumental instability. J. Toomre, F. Hill and L. November (Joint Institute for Laboratory Astrophysics, Boulder) presented evidence for a time-varying distortion of the modes that may be a result of the velocity and temperature fluctuations associated with giant cells, and it was indicated how such distortions might be used to measure the convective flow.

Five-minute oscillations of low degree

There are several discrepancies between the results of the whole-disk Doppler measurements made by G.R. Isaak (University of Birmingham, UK) and his colleagues³ and those by E. Fossat University of Nice, France and his colleagues⁴. Most striking is the separation of modes of like degree: the Birmingham group find an almost constant value of $135.2 \pm 0.2 \mu\text{Hz}$ in the frequency range 2.3 — 4.0 mHz, whereas Fossat and his colleagues find that the separation increases with frequency, in accord with theory, and has a mean value of about $136.0 \mu\text{Hz}$. Another point of disagreement concerns the lifetimes of the modes. From an analysis of sine-wave fits to data obtained on 28 contiguous days⁵, Isaak and his colleagues claim lifetimes of weeks, and are thus able to measure rotational splitting of $0.78 \pm 0.1 \mu\text{Hz}$ sidereal. On the other hand, the single continuous record of 120 hours duration obtained from the South Pole indicates lifetimes of only about two days. Thus Fossat and his colleagues doubt that rotational splitting of five-minute modes of low degree is even detectable.

Five-minute oscillations in the bolometric intensity of the Sun were

100 years ago

SOLAR PHYSICS

from Professor Stokes

At the conclusion of my last lecture I stated my belief that those changes which are continually going on at the surface of the sun had their origin in currents of convection, and I illustrated the processes which are there going on by what we know to be going on the surface of our own earth. I referred, but only historically, to a theory which was thrown out many years ago as to the origin of solar heat by Sir William Thomson, according to which it depended on the impact of meteoric bodies. I stated my belief that the spots were produced by the downward rush of, comparatively speaking, cool portions of gas which had been in the first instance ejected during these eruptions. In speaking to Mr. Lockyer afterwards I found that he had obtained independent evidence from his spectroscopic researches that these spots consisted of down-rushes of gas, and not, as some have supposed, of up-rushes.

From *Nature* 22, 613, October 27, 1881.

measured from the Solar Maximum Mission (H.S. Hudson, University of California). The relative amplitudes of the strongest modes were found to be 1.2×10^{-6} . The two frequencies where the power was greatest were roughly equidistant from corresponding values obtained from the two whole-disk Doppler measurements.

The discrepancies between the observations might appear to be small, but for diagnostic purposes the level of disagreement is substantial. One possible explanation of the difference between the results is that the Sun is changing, for the observations were made at different epochs. But before we can entertain this idea seriously, we should perhaps have in hand simultaneous observations made with the different instruments.

The 160-minute oscillations

In addition to the well known Doppler measurements, V.A. Kotov (Crimean Astrophysical Observatory) reported variations in infrared intensity with relative amplitude about 1×10^{-4} , in radio brightness with amplitude (centre-to-limb difference) 3×10^{-4} and in radio polarization with amplitude 1×10^{-4} . Hudson put an upper limit from SMM data of 5×10^{-6} in bolometric magnitude.

There now seems to be general agreement that the oscillation is solar, despite the fact that the period of 160.010 minutes is close to one ninth of a terrestrial day. Past fears that the oscillation is a manifestation of a terrestrial atmospheric phenomenon had been alleviated only slightly by the phase agreement between the independent observations at the Crimea, Pic-du-Midi and Stanford, because the observatories are separated by almost integral multiples of 40 degrees in longitude. These problems were avoided by Fossat, Grec and Pomerantz who observed the oscillations

from the South Pole⁴.

There was considerable debate over the nature of the oscillation. If it is essentially a linear eigenmode it must be a g mode, but why is only one of the densely distributed eigenmodes seen? One possibility is that resonant interactions with p modes is sufficiently selective. W.A. Dziembowski (Copernicus Astronomical Centre, Warsaw) discussed this idea, and argued that resonance is most likely with modes of high degree. Another possibility is that the mode is driven by nuclear reactions in the core, and that only one of the g modes is sufficiently concentrated near the centre of the Sun for driving to predominate over dissipation in the envelope. Nonlinearities have been invoked to concentrate the mode⁶, suggesting that it is a solitary wave propagating around the edge of the core. At the colloquium, E.A. Gavryuseva, Yu. S. Kopysov and G.T. Zatspin (USSR Academy of Sciences, Moscow) explained how linear g modes can also be concentrated, when the helium distribution is appropriate, in a manner reminiscent of an earlier discussion by W. Unno⁷. A.B. Severny and A.G. Kosovichev (Crimean Astrophysical Observatory, USSR) investigated the possibility that the oscillation is a product of interference between p modes, but it appears that the natural frequency that emerges is the mean separation between high order modes, which is measured to be $135.2 - 136.0 \mu\text{Hz}$ and corresponds to a period of only 123 min. Perhaps the most exotic suggestion was made by S.I. Blinnikov and M. Yu Khlopov, (USSR Academy of Sciences, Moscow) who maintained that the oscillation is a manifestation of the perturbation in gravitational field arising from a body of y-matter in orbit beneath the supergranular layer.

Despite the considerable attention that has been devoted to this oscillation, we are still far from a conclusive explanation.

Helioseismology

Finally, there was a discussion of how the frequencies of solar oscillations might be used to infer the internal stratification and rotation of the Sun. The p modes alone give information about only the very outer layers and a region in the envelope between radii of about $0.3R$ and $0.7R$, where R is the radius of the sun. However, it is likely that a measurement of the entire density stratification can be made to an accuracy of a few per cent with the addition of only a few f- and g-mode frequencies. These may be hard to obtain because the modes required are expected to have lower amplitudes than the p modes. R.M. Bonnet (Laboratoire de Physique Stellaire et Planétaire, Verrières-les-Buisson, France) concluded the meeting by reviewing the projects that have been proposed for obtaining the necessary data, the most promising of which appears to be a simple whole-disk Doppler measurement from space of the kind that was successfully made from the South Pole. □

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REVIEW ARTICLE

Microtubule treadmills—possible molecular machinery

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Microtubule polymerization in vitro is the summation of different reactions occurring at each end of the polymer. In steady-state conditions in vitro, net tubulin addition on to the microtubule occurs at one end of the polymer, and net tubulin loss occurs at the opposite end. Thus, a unidirectional flux of tubulin from one end of the microtubule to the other, or 'treadmilling', can occur. The opposite end assembly-disassembly behaviour of microtubules, if it occurs within cells, could be fundamentally linked to the functions of microtubules, as, for example, in the translocation of chromosomes during mitosis.

It has become evident during the past several years that the cytoplasm of eukaryotic cells is highly organized, and contains an elaborate and dynamic three-dimensional network of fibrous skeletal components. The major components include, at a minimum, microtubules, microfilaments, intermediate filaments and their associated structural and regulatory molecules.

Microtubules (Fig. 1) are 25-nm diameter hollow cylindrical polymers, found in virtually all eukaryotic cells. They provide the cell with a dynamic structural framework with which it may mould various morphogenetic changes, and with an apparatus for the movement of organelles from place to place within the cellular environment. In both these functions, microtubules sometimes appear to integrate with intermediate filaments and microfilaments (see refs 1–7 for reviews). The basic building block of the microtubule is the protein tubulin, which is a 6S dimeric 100,000 molecular weight protein composed of two non-identical ~50,000 molecular weight protein chains designated α and β (refs 8–12). Tubulin is a strongly conserved protein¹³, and one could consider the tubulin polymer to be a bare skeleton which has been adapted to specific tasks by association with a variety of proteins and cofactors^{14,15}, or by post-translational modification of the tubulin itself. The fact that there are many distinct tubulin genes^{16,17} presents a possible further dimension of regulatory complexity.

Microtubules can be grouped into subclasses based on their stabilities. Some microtubules, such as those found in cilia and flagella, are completely stable, and the functions of these microtubules are clearly not linked to their assembly-disassembly properties. Most microtubules in eukaryotic cells, however, are labile. This group includes most microtubules found in the mitotic spindles of dividing cells, those in the axons and dendrites of neurones and microtubules found as a disperse network in the cytoplasm of mammalian fibroblast cells. In general, labile microtubules are depolymerized at low temperatures, by high calcium ion concentrations and by the action of antimitotic drugs such as colchicine, podophyllotoxin and vinblastine^{1–7}.

It seems that a state of active equilibrium between microtubules and their subunits is often essential for the function of labile microtubules within cells. For example, the microtubules of mitotic spindles are in a state of dynamic equilibrium¹⁸ with their cytoplasmic pool of subunits. Microtubule disassembly seems to be essential for chromosome to pole movement during anaphase, with the rate of microtubule disassembly being rate-limiting for the movement (reviewed in ref. 19). Thus, the lability of spindle microtubules, insofar as it reflects the rates of

assembly and disassembly of the microtubules, seems to be linked in an important way to chromosome movement. In the same manner, the assembly and disassembly properties of other microtubules may also be linked to their functions.

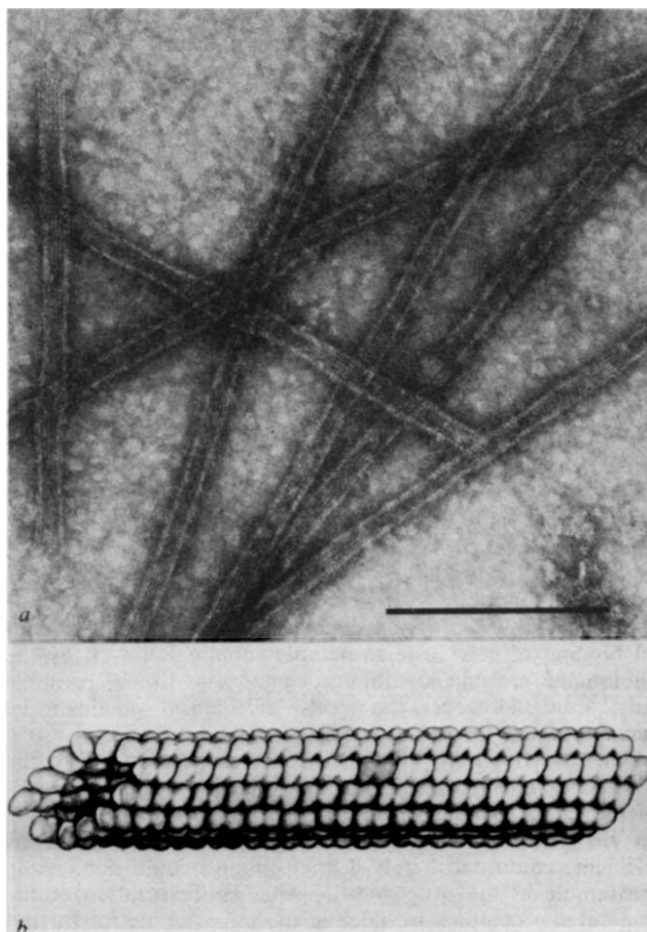


Fig. 1 Images of the microtubule structure. *a*, A negative stain whole mount preparation of purified bovine brain microtubules. The longitudinal filamentous substructure and the exclusion of stain by the walls of the tube are visible. The microtubules are ~25 nm in diameter. Scale bar, 0.2 μ m (micrograph by Dr Mary Ann Jordan). *b*, An idealized representation of the subunit structure of the microtubule. A single dimeric subunit is depicted in black on the microtubule. (Based on data of Dr L. Amos⁶. Drawing by Bio-Medical Illustrations, Seattle.)

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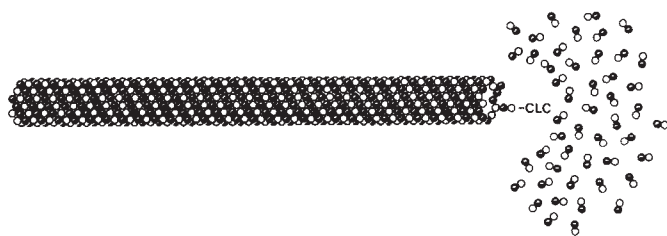


Fig. 2 A schematic representation of substoichiometric drug blockage of microtubule assembly. When only a small number of subunits is drug bound, assembly is strongly inhibited. If a free equilibrium of the drug-bound dimer with the microtubule end existed, assembly would be minimally inhibited by substoichiometric drug, because a large excess of drug-free subunits is available to displace the drug-bound subunit at the assembly site.

Drugs as biological probes for studying microtubule polymerization and function

The conditions for *in vitro* assembly of microtubules were discovered by Weisenberg²⁰, and since then the specific requirements and optimal conditions for *in vitro* polymerization of tubulin (primarily from brain, but also from non-neural cells and tissues) have been extensively studied. The assembly reaction has many of the properties of a nucleated helical polymerization of the type described by Oosawa and Kasai for the polymerization of actin²¹, with growth and shortening of the microtubule occurring by addition and loss of tubulin subunits at the ends of the polymer. Early studies by Allen and Borisy²² and by Binder *et al.*²³ established that growth at the two microtubule ends occurred at different rates, thereby demonstrating that microtubules possess an intrinsic growth polarity.

A number of drugs are now known to interfere with microtubule function in cells, and most act by inhibiting microtubule polymerization. Several of the drugs, such as colchicine and the vinca alkaloid, vinblastine, have been characterized as substoichiometric poisons^{24–26}. Tubulin has one high-affinity binding site for colchicine, and 50% inhibition of bovine brain microtubule polymerization occurs when only 2% of the tubulin free in solution is bound by the drug^{24,27}. There are two high-affinity binding sites on tubulin for vinblastine, both different from the colchicine site^{28–30}. This drug also inhibits the assembly of bovine brain tubulin by 50% when only a very small percentage of the tubulin free in solution is bound by drug. It seems clear that these drugs are not inhibiting assembly by binding stoichiometrically to soluble tubulin and inactivating the tubulin so that it is incapable of participating in the assembly reaction. Therefore, either the drugs alone, or drug-tubulin complexes, must be interacting with one or both ends of the microtubule to produce the inhibitory effect. With colchicine, microtubule assembly is inhibited by a mechanism that involves the sequential binding of colchicine to soluble tubulin followed by the binding of colchicine-tubulin complexes to microtubule ends^{24,27,31,32}. However, the precise mechanism remains to be elucidated.

Our investigations into the underlying reasons for the substoichiometric poisoning of microtubule assembly by colchicine led us to the discovery of steady-state tubulin flux. We felt that substoichiometric blockage of polymerization by colchicine could occur only if microtubules could not readily disassemble at the drug addition site, as dissociation of the drug-tubulin complex would free the microtubule for further assembly due to the large excess of available free tubulin (shown schematically in Fig. 2). We reasoned that one of the possible explanations for retention of colchicine-tubulin complex at the ends of the microtubules was that at apparent equilibrium, subunits were being gained predominantly at one end of the microtubule, and were being lost predominantly at the opposite end³³.

Microtubules as molecular treadmills: steady-state microtubule flux

To determine whether there was a net addition of tubulin at one end of the microtubule (this would be designated the net assembly end, or A-end; see Fig. 3) and a net loss at the other end (the net disassembly or D-end; Fig. 3) at apparent equilibrium, we took advantage of the observation that guanine nucleotide (GTP) which binds to an exchangeable site on tubulin when the tubulin is free in solution, becomes non-exchangeable (as GDP) when the tubulin becomes incorporated into the polymer^{34–36}. Thus, we found that bovine brain microtubules assembled to apparent equilibrium and exposed to a ³H-GTP pulse incorporated the label linearly during a long time course, provided that care was taken to maintain the GTP pool in the presence of the normal rapid hydrolysis by inclusion of a GTP-regenerating system (Fig. 4). Microtubules assembled initially in the presence of tritiated nucleotide to label their entire length, lost label in a linear manner during an unlabelled GTP chase with the same time course as label was gained during a steady-state pulse³³.

Our interpretation of the linear loss and gain of guanine nucleotide was that, at apparent equilibrium *in vitro*, subunits were added in a net manner at one end of the microtubule and lost at the other end. By determining the mean microtubule length we could calculate the rate of tubulin flux in the conditions used, and for steady-state bovine brain microtubules, the flux rate was 0.7 $\mu\text{m h}^{-1}$.

Several kinds of experiment could be performed to characterize further the tubulin flux and to eliminate possible alternate interpretations of the data, such as the possibility that label loss and gain represented guanine nucleotide exchange along the length of the polymer, rather than label loss and gain at the ends of the polymer. For example, podophyllotoxin, a drug which acts like colchicine, blocked label uptake but did not appreciably affect label loss from fully labelled microtubules. Furthermore, label uptake was shown to be proportional to the microtubule number concentration. Further, if net tubulin incorporation occurred at one end and net loss occurred at the other end, one would predict that a pulse of ³H-GTP would be retained in the microtubule during a GTP chase until the tubulin dimer to which the labelled nucleotide was bound transited from the A-end where it became incorporated, to the D-end where it would be lost (see Fig. 5). Figure 6 shows the result of such an experiment, in which microtubules were assembled to apparent equilibrium in unlabelled nucleotide. They were sheared to an average but disperse length of 3.5 μm , pulsed for 30 min with ³H-GTP and then chased for 6 h with unlabelled GTP. No label was lost until after the first 90 min of chase (that is, no microtubules were shorter than 1 μm) and with increasing time progressively more label was lost from the microtubules. Label

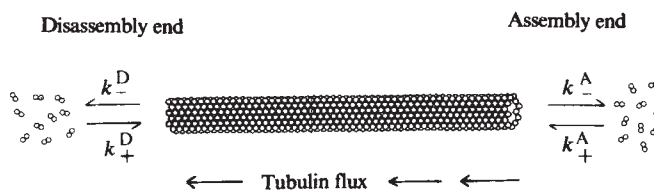


Fig. 3 Steady-state reactions at microtubule ends. Equilibrium reactions exist at each microtubule end. At steady state, the rate constant for net tubulin addition at one end (k_A^+) is greater than the rate constant for net tubulin loss (k_A^-). This end is designated the 'net assembly', 'A-end' or 'assembly end' of the microtubule. The rate constant for net tubulin loss at the opposite end (k_D^-) is greater than the rate constant for net tubulin addition (k_D^+). This end is designated the net disassembly, D-end or disassembly end of the microtubule. At steady state, there is a precise balance of net subunit gain at the A-end with the net subunit loss at the D-end, resulting in the unidirectional flux of tubulin from the A-end to the D-end of the microtubule. We are unable to use the plus (+) and minus (-) designations suggested by Bergen and Borisy³⁹ for identification of the individual ends of the microtubules. The plus and minus assignments are based on flagellum-seeded growth of tubulin, and the relationship between the plus and minus ends of these microtubules, and the net assembly and disassembly ends of steady-state bovine brain microtubules, has not been established.

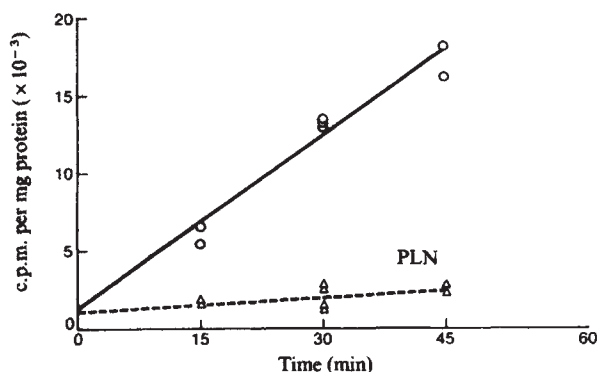


Fig. 4 Nucleotide incorporation into microtubules at steady state. Bovine brain microtubules assembled to steady state in the presence of assembly buffer, 0.1 mM GTP and a nucleoside triphosphate-regenerating system, were assayed for incorporation of a ^3H -GTP pulse by methods described elsewhere³³. In the absence of any assembly-inhibitory drug, label uptake into the polymer is apparently linear with time. In the presence of sufficient podophyllotoxin (PLN) to block assembly completely, no label is incorporated. Disassembly of the polymer in PLN is occurring slowly ($\sim 7\% \text{ h}^{-1}$).

remaining in the microtubules at the end of the 6 h was due to the presence of some microtubules which remained unshredded (9–10 μm long). This experiment clearly demonstrates the head-to-tail assembly or 'treadmilling' which occurs in this system at steady state *in vitro*.

Further, and of considerable importance, it was shown that treadmilling is a mechanism intrinsic to the tubulin backbone of the microtubule, as microtubules reassembled from pure sea urchin sperm tail outer doublet tubulin also exhibited the same treadmilling behaviour observed with bovine brain microtubules¹⁵. Purified bovine brain microtubules are composed of ~ 25 –30% microtubule-associated proteins (proteins that co-purify with microtubules during cycles of warm assembly and cold disassembly), whereas outer doublet tubulin, to the limits of electrophoretic resolution, is free of microtubule-associated proteins.

The method we used to characterize opposite-end assembly and disassembly of microtubules at steady state measured only the rate of steady-state flux of tubulin which had become incorporated into the polymer at the net A-end. The method could reveal nothing about the molecular association and dissociation events which reflect the reversible equilibrium reactions occurring at each end of the microtubule. It is now clear that there are distinctly reversible equilibrium reactions at each microtubule end^{15,37–44} (shown schematically in Fig. 3). For example, Karr and Purich³⁸ devised a rapid dilution technique which enabled them to study the depolymerization kinetics in non-steady-state (dilution) conditions. Their results indicated that tubulin release from the net assembly end in dilution conditions is very rapid. Bergen and Borisy have determined the rate constants for tubulin addition and loss at the two ends of the microtubule by measuring the growth, by electron microscopy, of porcine brain microtubule protein on to the proximal and distal ends of stable flagella outer doublet microtubules isolated from *Chlamydomonas reinhardtii*³⁹. They assumed in their study that the outer doublet microtubules served as an internal marker of polarity and did not alter the polymerization kinetics characteristic of the two ends of a microtubule. These investigators have found that net elongation (growth) is more rapid on the distal end of the flagellum outer doublet microtubules (referred to as the plus (+) end) than on the proximal (minus (-)) end. In the conditions used in their study, Bergen and Borisy found that both ends of the microtubule displayed dynamic activity. Further, their data demonstrated by a method independent of the one used by us³³ that microtubules possess an intrinsic head-to-tail assembly and disassembly behaviour. The rate of tubulin flux obtained in the conditions used by Bergen and Borisy was 3–6 $\mu\text{m h}^{-1}$, which is considerably more rapid than the 0.7 $\mu\text{m h}^{-1}$ flux rate obtained with steady-state bovine brain

microtubules *in vitro*³³, or with steady-state microtubules obtained from sea urchin outer doublet tubulin¹⁵.

However, in the conditions used, Bergen and Borisy found that flux was very inefficient. Their method permitted calculation of the rate constants for gain and loss of tubulin, and they found that on the average, there was a lengthening of the microtubule by one subunit at the growing (+) end, and a shortening by one subunit at the (-) end, for each 14 association and dissociation steps. Thus, the extent to which dissociation of tubulin at the A-end of a microtubule competes with steady-state net tubulin addition may be very considerable, leading, in certain conditions, to a very low ratio of subunits actually becoming incorporated and fluxing, as compared with the number of subunits associating and dissociating at that end. More recent experiments by Cote and Borisy⁴⁰ and by Cote *et al.*⁴¹, in which association and dissociation rate constants and steady-state tubulin flux have been measured with pure 6S tubulin, have found considerably more efficient flux than found in the study just described, and a flux rate of $\sim 50 \mu\text{m h}^{-1}$ in steady-state conditions *in vitro*. This flux rate is approximately equal to the rate of chromosome movement during anaphase (see below).

Margolis³⁷ has recently observed that the efficiency of tubulin flux with steady-state bovine brain microtubules *in vitro* can vary considerably depending on the concentration of GTP. For example, at high GTP concentration (1.0 mM), the treadmilling incorporation of tubulin was relatively inefficient, in that a low percentage (21%) of the tubulin dimers that bound to the A-end actually become permanently incorporated at that end, whereas at low GTP concentration (0.1 mM), the treadmilling incorporation was $\sim 80\%$ efficient.

The existence of treadmilling in steady-state conditions requires that the critical tubulin concentration for assembly at the net A-end be lower than the critical concentration for assembly at the net D-end (see discussion in ref. 43). As would be expected of a steady-state system, it is now clear that energy provided by GTP hydrolysis is expended to maintain the disparity in critical concentration at the two microtubule ends, and, therefore, to maintain treadmilling. In the absence of GTP hydrolysis, tubulin flux does not occur^{37,44}.

If microtubule treadmilling can occur in cells, it is reasonable to expect it to be under homeostatic regulation. We have found that the treadmilling rate of bovine brain microtubules *in vitro* is strongly responsive to the addition of ATP, speeding up as the ATP concentration is increased⁴⁵. A cyclic AMP-dependent protein kinase activity that phosphorylates microtubule-associated protein 2 (MAP2) may be responsible, although phosphorylation of other microtubule-associated proteins may also be involved (R.L.M., K. F. Sullivan, C. Rauch and L.W., in preparation). Jameson and Caplow have also observed an increase in the tubulin flux rate when phosphorylated MAPs are

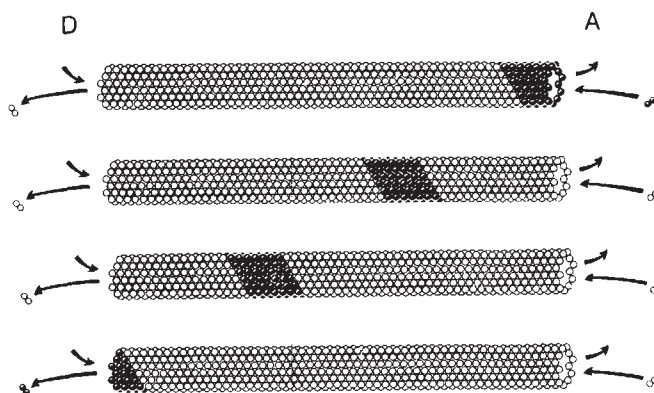


Fig. 5 Schematic illustration of the migration of a short ^3H -GTP pulse representing incorporated tubulin (black subunits) from the net assembly end (A-end) to the net disassembly end (D-end) of a steady-state microtubule. During a brief chase with unlabelled GTP (represented by white subunits), all label would be retained. During a long chase with unlabelled GTP, all label would be lost.

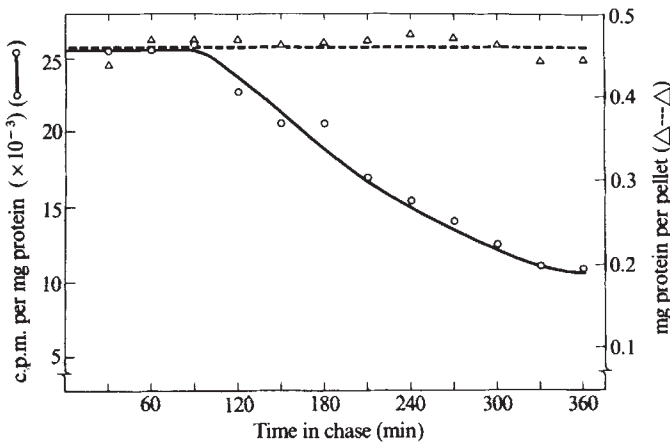


Fig. 6 Demonstration of subunit treadmilling through microtubules by a pulse-chase experimental procedure. Sheared steady-state bovine brain microtubules (average length $3.5 \mu\text{m}$) were pulsed with ^3H -GTP, in conditions described in Fig. 4 legend, for 0.5 h. Following the pulse, microtubules were chased with 30-fold excess GTP for the times indicated. Label and protein retained in the polymer were assayed by analysis of microtubule pellets isolated at the end of the experiment by centrifugation through a 50% sucrose cushion. $\circ$, ^3H -GTP; $\triangle$, protein in pellet.

reconstituted with 6S tubulin as compared with reconstitution of tubulin with non-phosphorylated MAPs⁴². Thus, modulation of tubulin flux by a cyclic AMP-dependent protein kinase through phosphorylation of MAPs may be part of a regulatory system affecting microtubule function in cells. Interestingly, depletion of the cellular ATP pool reduces the rate of microtubule disassembly in cells, whereas addition of ATP increases the disassembly rate⁴⁶, suggesting that an ATP-dependent reaction controls microtubule disassembly in cells.

In summary, the phenomenon of steady-state head-to-tail microtubule polymerization and depolymerization has been documented by independent methods in several laboratories^{15,33,37,42,44,45}. The possible consequences of this phenomenon with microtubules in cells is that a constant subunit flow from one end to the other of an unconstrained microtubule could occur, thus creating a molecular treadmill with the potential for doing work to move organelles or other attached materials from place to place.

Possible cellular mechanisms and functions of treadmilling

As exemplified by flagellar outer doublet microtubules, microtubules appear to have mechanisms for suppressing treadmilling and stabilizing themselves, despite the fact that their constituent subunits can be shown to form treadmilling polymers *in vitro*¹⁵. The fact that a polymer can treadmill in the test tube does not mean it inevitably will; rather, it is likely that the phenomenon is subject to cellular controls so that it occurs in the proper context, to fit a particular role.

Treadmilling, as observed by Kirschner⁴³, may be an *in vitro* manifestation of a molecular mechanism that has other purposes in the cell. Kirschner has suggested that microtubules and also actin filaments use GTP and ATP hydrolysis, respectively, to produce a disparity in the critical subunit concentration for assembly at the two ends of the polymers, so that the growth of unanchored polymers is suppressed. For example, if a microtubule were anchored in an organizing centre at the D-end, and if that end were not free to exchange subunits (a requirement of the Kirschner model), then the addition and loss of tubulin to that microtubule would occur only at the A-end, and would be determined by the critical subunit concentration for assembly at that end. If a microtubule were unanchored, with the D-end free for subunit exchange, and if the critical concentration for net assembly at the D-end were appreciably higher than the free subunit concentration in the cell, then the unanchored microtubule would not survive. This mechanism, then, uses the

hydrolysis of GTP to restrict microtubule assembly to specific seeded sites.

Two aspects of the Kirschner model should be emphasized. First, the model requires that an anchored microtubule end is not free to exchange subunits. Thus, the anchorage at a kinetochore or a centrosome completely 'caps' the end of the microtubule so that no gain or loss of tubulin can occur. Second, all the microtubules within the cytoplasm of a specific cell would be expected to be anchored at the same end (for example, all anchored at the D-ends), with all free (assembly and disassembly competent) ends the same (for example, all A-ends free).

Another possibility is that microtubule treadmilling could be used by the cell to produce work, as in the translocation of one object past another. In this context, there are three important

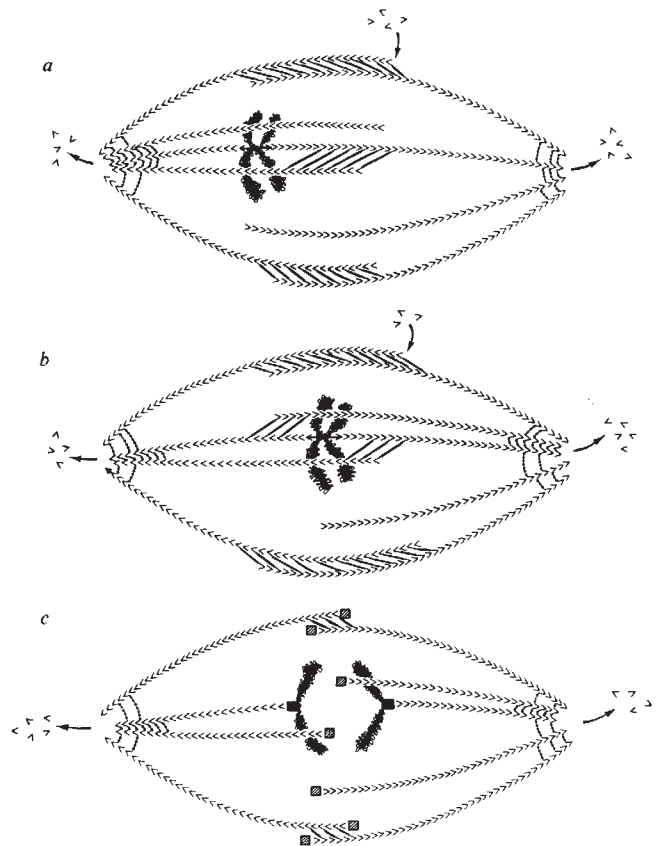


Fig. 7 A schematic representation of our proposed mitotic mechanism^{58,59}.

The spindle region of a typical higher eukaryotic mitotic apparatus is depicted. Arrow lines indicate microtubules. The arrows represent the direction of dimer flow in the microtubules created by steady-state assembly-disassembly dynamics and by the sliding of antiparallel microtubules past each other. Microtubules are parallel or antiparallel depending on whether their arrows point towards the same or opposite poles, respectively. Those microtubules that attach to chromosomes are kinetochore-to-pole (k-p) microtubules, all others are interpolar (i-p) microtubules of various lengths, which originate at or near one pole and traverse the spindle. The kinetochore, the organelle by which k-p microtubules attach to chromosomes, is represented by a line through the metacentric chromosome constriction. *a*, In prometaphase there is a re-establishment of microtubule assembly, following a transitional assembly blockage that permits the dismantling of the interphase microtubule network, and the establishment of organizing centres for the mitotic apparatus. Antiparallel microtubule sliding at this stage causes chromosomes to migrate towards the metaphase plate. *b*, At metaphase microtubules are at steady state. The mitotic apparatus structure is maintained by the internal tension of antiparallel i-p microtubule sliding that pulls on sister chromatid linkages. *c*, Anaphase is characterized by the cleavage of sister chromatid links, and by simultaneous blockage of microtubule assembly at the kinetochore (solid blocks) and possibly, but not necessarily, at the equatorial region (hatched blocks), while i-p microtubule sliding continues. Note that k-p microtubules need never be actually treadmilling in order to be acted on by the spindle; however, there must be a special k-p anaphase disassembly mechanism (see Fig. 8), as these microtubules are often seen to shorten and yet remain attached at both ends. Although parallel microtubule linkages are depicted as providing pericentromeric convergence, linkage to lateral anchoring elements at the poles (see Fig. 8) may also serve this capacity. $\bullet-\bullet-, Parallel microtubule linkages; $\nearrow$, antiparallel sliding linkages between microtubules.$

questions to consider in order to model any role treadmilling may have in a particular microtubule-dependent mechanism. First, what are the orientations of the A- and D-ends with respect to microtubule-organizing centres, or transported constituents; second, are both microtubule ends free to exchange subunits; and third, by what mechanism can the polymer be anchored and translocated in the cell so that its assembly reaction can do work?

With respect to the question of microtubule polarity, Heide-mann and McIntosh⁴⁷ and Euteneuer and McIntosh^{48,49} have recently developed an important technique which makes it possible to determine the orientation of microtubules in cells. They have used special buffer conditions to produce hook-like projections composed of tubulin that project from the surface of intact microtubules. When viewed in cross-section, the hooks rotate in a clockwise or anticlockwise manner depending on the polar orientation of the microtubules (viewing towards or away from a (+) or (-) end, according to the polarity designation of Bergen and Borisy³⁹). Pre-existing microtubules can be decorated in this way, and their polarity determined⁴⁹. For example, in the mitotic spindles of rat kangaroo PtK₁ cells⁴⁹, all microtubules belonging to a half-spindle (both kinetochore and interpolar microtubules) appear to be oriented with the same polarity. Telzer and Haimo⁵⁰ obtained similar results using spindles isolated from eggs of the surf clam *Spisula solidissima*. These investigators have shown that the spindle microtubules could bind dynein from axonemes of *Tetrahymena thermophila* in a way that revealed the relative polarity of the microtubules. Their results, in accord with those of Euteneuer and McIntosh⁴⁹, indicate that all the microtubules in a half-spindle possessed the same polarity. The two half-spindles, therefore, represent two sets of microtubules that may interdigitate in an antiparallel manner with microtubules of the opposite half-spindle. If steady-state net disassembly ends (D-ends as we have defined them) of the microtubules within each half-spindle are oriented towards the poles, this would be the orientation consistent with our model of mitotic chromosome movement based on microtubule treadmilling (see Fig. 7, discussed below).

It is important to point out, however, that the microtubule orientation predicted by the above results is the opposite of that expected on the basis of *in vitro* growth studies with microtubules and various initiating complexes⁵¹⁻⁵⁶, and of that predicted by the Kirschner model⁴³ (unless kinetochores can capture elongating microtubules as proposed by Euteneuer and McIntosh⁴⁹). *In vitro* microtubule growth experiments using centrosomes and kinetochores as nucleating sites *in vitro* have indicated that all the microtubules growing from these sites have their fast growing ends ((+) ends in the terminology of Bergen and Borisy³⁹) distal to the site of initiation. It is too early to explain the disparity between the *in vivo* and *in vitro* data, but as pointed out by Euteneuer and McIntosh⁴⁹, the *in vitro* results involve a more or less severe disruption of the *in situ* morphology and might be displaying a misleading result.

An anchorage-translocation system

It is worth considering what the requirements would be in order that a unidirectional tubulin flux in microtubules might be used to do work, such as in the translocation of chromosomes during anaphase of mitosis. Tubulin flux in microtubules, or treadmilling, can occur in the cell if both ends of a microtubule are free to exchange subunits. However, inspection of electron micrographs of microtubules emanating from kinetochores or pericentriolar regions of cells suggests that all the microtubules have at least one end embedded in a structural matrix of some kind⁵⁷. It is, however, conceivable that microtubules are attached to a structural matrix by lateral linkages near their ends, in such a way that the ends themselves remain free for subunit addition or loss (see Fig. 8 legend for further discussion). This possibility is excluded in the Kirschner model as discussed previously⁴³.

If both the assembly and disassembly ends of microtubules are free to exchange subunits, it is reasonable to ask how the

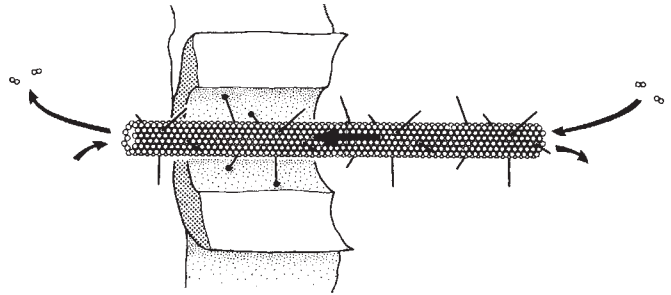


Fig. 8 Proposed lateral linkage element for microtubules. For microtubules both to treadmill and to remain apparently attached by their ends to cell organelles such as kinetochores and pericentriolar regions, a special mechanism for translocation past these fixed objects is necessary. That such a translocation occurs is evident in anaphase when kinetochore-to-pole microtubules, firmly embedded at both their ends in the kinetochores or the pericentriolar regions, nonetheless disassemble in an orderly manner without detaching either end. In fact, because these microtubules transmit the poleward forces to the separating chromatids, their attachment at both ends while disassembling appears necessary to their function. If the same lateral dynein-like linkages that we have proposed for separate antiparallel microtubules (Fig. 7) to allow poleward displacement against fixed lateral elements, simultaneously the microtubule ends would be free for assembly-disassembly reactions, the microtubules would translocate unidirectionally, and would remain firmly attached to microtubule-organizing centres. Further, since a unique enclosed environment could be created beyond the lateral linkages, a specialized disassembly mechanism (possibly calmodulin dependent)^{81,82} might disassemble only that portion of microtubules which has translocated past lateral linkages. Such a lateral linkage element could also operate at the kinetochore, to produce constant outward translocation of the microtubule as it assembles. Similar attachment-translocation sites might be involved wherever microtubules insert into fixed organelles. A cut-away drawing is shown which is meant to demonstrate not so much what such an organelle might look like, but rather how it might operate. The microtubule has dynein arm projections that attach and translocate unidirectionally by rowing past this enclosing element (in the direction of the large arrow). Net assembly and disassembly ends, as depicted, remain free to exchange subunits.

polymers might be anchored and in what way they may push against fixed objects in order to translocate and thereby transform treadmilling into work. We have previously proposed a model of treadmilling utilization in mitosis, in which each of the two half-spindle sets of microtubules (each set emanating from a pole) interacts with the opposing half-spindle system to drive it towards its pole at a rate that just matches and compensates for the rate of growth of microtubule A-ends^{58,59}. A constant equatorial assembly (in the region of overlap between the two half-spindle systems) and poleward flow of microtubules is thus established, which can be coupled to the chromatids at the proper times so as to segregate them poleward in anaphase (Fig. 7). The model suggests that the disassembly ends remain free to dissociate subunits while the microtubule bundles aggregate towards and appear tethered to the pericentriolar locus. The *in vivo* polarity determinations of Euteneuer and McIntosh⁴⁹ and Telzer and Haimo⁵⁰ are consistent with the proposed mitotic model.

The microtubule translocation past fixed objects (for example, components of the pericentriolar matrix, organizing centres or even kinetochores) that is necessary to convert tubulin flux into work, could involve rowing past these objects by the making and breaking of dynein-like cross-bridges. As these interactions, although creating firm anchorage, are on the lateral surface of the microtubule, the microtubule ends remain free to exchange subunits (Fig. 8). Viewed in this way, the microtubule backbone itself may have a passive role in the anchorage and translocation, with the active work-producing machinery functioning laterally along the surface of the microtubule.

Microtubules could be used in the cell in a highly versatile manner if they were functioning as rigid or semi-rigid passive rods, but with the potential for coupling the assembly, disassembly or flux of tubulin to an anchorage-translocation array of machinery. At one end of the spectrum of functional possibilities, the microtubule could be modified by associated proteins to create a stable rigid rod, and the function of the microtubule would be mediated by an anchorage-translocation

array of machinery not coupled to assembly, disassembly or flux (as in the case of sperm flagella microtubules). At the other functional extreme, the microtubules would be capable of treadmilling. The rate of treadmilling, and translocation of cell components which are attached to the microtubule surface, would be strictly coupled to the work-producing anchorage-translocation machinery. Between these extremes a wide variety of mechanisms could be envisaged. For example, work-producing anchorage and translocation machinery may be coupled with disassembly near the D-end of a microtubule. In this situation, a microtubule could ratchet past a fixed anchorage-translocation complex concomitant with depolymerization, while the cell components attached to the microtubule at or near the A-end would move towards the fixed anchorage-translocation complex. Such a mechanism may be functioning in the case of kinetochore microtubules during anaphase^{58,59}.

We visualize a treadmilling mechanism as being of use to the cell in at least two ways: to translocate organelles or other substances from place to place, and to act as a temporal and spatial marker for the various morphological changes that are coordinated with the microtubule system. Provided that microtubules could flow past fixed objects in the cell, they would be able to translocate any vesicles or other organelles bound to their surface. The rate of translocation would equal the rate of microtubule treadmilling. In this way, microtubules could use treadmilling to translocate secretory granules, viruses or mitochondria, or maintain cytoskeletal systems such as melanosomes and intermediate filaments in a dynamic and readily reversible structure. Such translocations could conceivably occur over relatively great distances, as in microtubule-dependent slow axonal transport. Note that treadmilling is not the only conceivable means of transporting materials. Microtubules may also remain stationary and translocate organelles along their length using dynein-like mechanisms⁶⁰, as previously indicated. It is possible that microtubules can form the passive skeletal framework on which an active transport system is independently constructed. This may be the case in the axopodia of heliozoans⁶¹, and has been suggested as an alternative mechanism in axonal transport⁶².

Microtubules often seem to affect events at a distance in the cell in a precise temporal and spatial manner. For example, during mitosis, the onset of anaphase (the breakage of chromatid bonds), the deposition of the cytokinetic furrow material and the onset of cytokinesis are all dependent on functioning microtubules and appear blocked if microtubules are absent. At low colcemid (an analogue of colchicine) concentrations the progress of anaphase and cytokinesis are coordinately delayed⁶³. It is possible that the microtubule treadmill acts as a timing mechanism in coordinating cellular processes. Indeed, as the placement of the cytokinetic furrow depends on the mitotic apparatus orientation, treadmilling may also communicate spatial information⁶⁴.

As a model of how such a mechanism might operate, consider the enzyme-catalysed addition of tyrosine to the carboxyl terminus of the α -subunit of tubulin⁶⁵. Whereas the favoured substrate for tyrosination is the subunit form of the protein⁶⁶⁻⁶⁸, the favoured substrate for the enzymatic removal of tyrosine is the microtubule⁶⁶⁻⁶⁸. Suppose the titre of detyrosinated tubulin activates cellular processes, and that the tyrosination reaction is blocked or slowed at the onset of mitosis. Then as mitosis proceeds, tubulin enters microtubules, is detyrosinated and released at the poles. Detyrosinated tubulin then diffuses outwards from the poles in ever increasing concentration and, when certain titres are reached, triggers anaphase cleavage of chromatids, deposition of cytokinetic material and, finally, cytokinetic ring contraction. If treadmilling is slowed by drug treatment, the signalling system would therefore be delayed to the same extent.

Even if the tyrosination-detyrosination cycle does not have such a role, it illustrates how cellular events could be timed relative to the amounts of tubulin polymerized in microtubules, and to the rate of microtubule treadmilling.

Treadmilling of actin filaments

As with tubulin, it has been known for several years that ATP hydrolysis is not essential for the polymerization of actin, yet hydrolysis of ATP accompanies the polymerization^{69,70}. Further, the addition of subunits occurs at the two ends of an actin filament at markedly different rates. The two ends of the actin polymer can be distinguished by decoration with heavy meromyosin 'arrowheads', and the rate of growth at the 'barbed' end is substantially greater than that at the pointed end⁷¹⁻⁷³. The results of Wegner^{74,75}, who studied the incorporation of labelled actin subunits into steady-state filaments, have indicated that the hydrolysis of ATP produces a disparity in the critical concentration for actin assembly at the two ends of the polymer, and as in the case of microtubules, a steady-state unidirectional net flow of actin subunits from one end to the other could occur in the presence of ATP. Kirschner's model⁴³, which proposes that nucleotide hydrolysis results in the selective stabilization of anchored filaments, applies to actin microfilaments in cells as well as to microtubules. Very limited data are available on *in vitro* head-to-tail assembly of actin filaments; however, Pollard and Mooseker⁷⁶ have analysed by electron microscopy the growth rates of actin onto bundles of actin filaments isolated from intestinal microvilli, and have obtained rate constants allowing the calculation of a steady-state actin flux rate of 0.7 molecules of actin per s in one of the sets of conditions used. Independent evidence that actin treadmilling can occur *in vitro* has obtained both directly⁷⁷ and indirectly^{78,79}. For example, using a fluorescent analogue of ATP as a probe for actin addition to and loss from steady-state actin filaments *in vitro*, Wang and Taylor⁷⁷ have measured a rapid subunit flux.

Several technical problems exist with microfilaments that do not seem to occur with microtubules. For example, the breaking and re-annealing of actin filaments which are difficult to measure could significantly hamper the interpretation of actin treadmilling data. As with microtubules, there is no evidence regarding the question of whether actin filaments can actually treadmill within cells. However, a recent report by Tilney *et al.*⁸⁰ has indicated that, despite membrane association of actin polymers at their barbed ends, assembly of actin filaments can occur at these ends. This observation makes active treadmilling of anchored polymers in living cells a more realistic possibility.

Concluding remarks

It is now well established that microtubules exhibit steady-state opposite end assembly and disassembly *in vitro*. Evidence which is not as extensive also indicates that actin filaments exhibit the same flux behaviour in the presence of ATP. It is important to emphasize that treadmilling has not been directly demonstrated in the cell, and until a direct demonstration has been made, it would be prudent to regard treadmilling as an *in vitro* phenomenon whose intracellular role remains to be established. If treadmilling occurs in the cell, it could be utilized in a great many ways to create and organize the flow of the cell substructure.

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ARTICLES

Compact radio source 1413+135 is a far-IR extragalactic object

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The radio source 1413+135 is shown to be one of the strongest known emitters of millimetre radiation. The energy distribution of this object measured between metre and X-ray wavelengths reveals that most of the power emitted by this object comes out at millimetre and far-IR wavelengths. If the emission at 1 mm is due to incoherent synchrotron radiation, then the source must be very compact, with a size around 25 μ arc s, and contain a magnetic field larger than 10 G. The steep spectral index in the near-IR is attributed to a high-energy cutoff in the distribution of synchrotron emitting electrons at a Lorentz factor $\gamma \sim 300$.

CONDON *ET AL.*¹ obtained an interferometric position for the moderately bright (0.7 Jy at 2.7 GHz¹), flat-spectrum radio source 1413+135, also known as OQ+122 (ref. 2). They were unable to find an optical counterpart to the source brighter than $m_v \sim 19$ mag on the Palomar Sky Survey prints. Rieke *et al.*³ examined the position of this 'empty field' source and found a relatively strong source of 2.2- μ m radiation with a very steep 1.25-2.2 μ m spectral index, $\alpha_{JK} = -3.4$ (where the spectral index α is defined by $F_\nu \propto \nu^\alpha$). Rieke *et al.* suggested that 1413+135 might be a new type of extra-galactic radio source based on the extreme steepness of its near-IR continuum. Beichman *et al.*⁴ extended the observations of 1413+135 to 10 μ m, obtained a near-IR spectrum and concluded, on the basis of the shape of the 1.2-10- μ m continuum, the lack of

features in the spectrum and the rapid variability at 2.2 μ m (ref. 3), that 1413+135 was an extremely red BL Lac object, emitting much of its power in the IR.

Observations

New measurements of 1413+135 at various wavelengths are presented in Table 1. Millimetre observations of 1413+135 were made using an incoherent liquid ⁴He cooled composite bolometer⁵⁻⁷ at the prime focus of the 5-m Hale telescope at Palomar Mountain. The 1 mm continuum observations of 1413+135 were followed immediately by observations of Jupiter and 3C273 at nearly the same airmasses. The amount of precipitable H₂O was determined by measurements of the

Table 1 Observing log for 1413 + 135

Date	Wavelength	Flux Density (mJy)
25–27 November 1980	1.0 mm	4,800 ± 500
4 December 1980	2.8 cm	2,300 ± 300
21–24 December 1980	1.0 mm	4,300 ± 400
22 December 1980	2.8 cm	2,100 ± 100
8 January 1981	2.8 cm	2,000 ± 100
17–21 January 1981	1.0 mm	4,300 ± 300
20 January 1981	2.4 mm	7,000 ± 2,000
15 March 1981	3.5 μ m	11 ± 1
	2.2 μ m	2.8 ± 0.13
	1.65 μ m	0.91 ± 0.05
	1.25 μ m	0.34 ± 0.03
23 March 1981	1.0 mm	6,100 ± 1,500
15 April 1981*	2.1 cm	3,500 ± 200
	3.75 cm	2,100 ± 200
22 April 1981	100 μ m	1,400 ± 1,000

* H. Aller and M. Aller, personal communication.

amount of H₂O along the line of sight to the Sun⁸. Jupiter was adopted as the primary flux standard using a brightness temperature of 168 ± 8 K (ref. 9). All the observations of 1413 + 135 were reduced to give a flux density at 1.0 mm (ref. 10), assuming a spectral index of 0. For $\alpha < 2$, the results are insensitive to the choice of α . The absolute uncertainty in the millimetre calibration is ~30%. The data were averaged to give a 1.0-mm flux density of 4.5 Jy; there was no evidence for variability greater than the statistical uncertainty in each observation.

New near-IR observations were obtained on 15 March 1981, using an InSb detector at the 5-m telescope.

Observations at 2.4 mm were made on 20 January 1981, using a SIS heterodyne receiver¹¹ with a 175 K noise temperature (double sideband) at the bent Cassegrain focus of the 10-m antenna at the Owens Valley Radio Observatory. The observations were calibrated relative to Jupiter for which a brightness temperature of 179 ± 5 K was used¹².

Observations at 2.8 cm were made on 4 December 1980 and on 8 January 1981, using the 40-m telescope at the Owens Valley Radio Observatory⁴. A flux density at 2.8 cm of 2.1 ± 0.2 Jy was obtained in both December 1980 and January 1981, with no evidence for variability. Aller and Aller (personal communication) report, however, that a large outburst occurred at 2.1 cm during September and October 1980.

Observations were made at 100 μ m using the Kuiper Airborne Observatory on 22 April 1981, with the photometer described by Harvey¹³. The observed flux density was 1.4 ± 1.0 Jy, corresponding to a 3 σ upper limit of 3.0 Jy. Observations made at 2 and 4 cm during mid-April (H. Aller and M. Aller, personal communication) shown that 1413 + 135 was not flaring at this time.

Discussion

The composite energy distribution for 1413 + 135 from 2 cm to X-ray wavelengths is shown in Fig. 1. The object 1413 + 135 varies at many wavelengths^{3,4,14} making it difficult to construct an overall energy distribution for the source. The new data shown in the figure were obtained from November 1980 to April 1981, but an observation at 2.8 cm was made contemporaneously with each of the other measurements and showed no variation >40% at these times. On the scale of Fig. 1 and for a discussion of the overall energetics of the source, these variations are not significant.

The observations at wavelengths longer than 100 μ m are plotted as obtained. An observation of 1413 + 135 at 10 μ m obtained in July 1980 has been incorporated into Fig. 1. A comparison of the July 1980 near-IR data⁴ and the March 1981 data show a brightening of about 40% during this time. Because

the shape of the energy distribution of 1413 + 135 shows little variation as the source brightness varies⁴, the July 1980 10- μ m data have been increased by a factor of 1.4 for inclusion in Fig. 1.

What distinguishes 1413 + 135 from other sources is its concentration of energy at millimetre and submillimetre wavelengths. More than 90% of the total luminosity of 1413 + 135 is emitted between 1 mm and 3.5 μ m. The spectrum of the source from 2 cm to 10 μ m resembles those of III Zw 2, a luminous Seyfert galaxy^{15,16}, and OJ287, a highly variable BL Lac object¹⁷, but declines much more rapidly at shorter wavelengths than in these objects.

Based on the 2.4- and 1.0-mm data of 19 January 1981 and the 2.8-cm data of the previous fortnight, it is apparent that the flux density distribution of 1413 + 135 has a maximum around a wavelength of 2.4 mm, with $\alpha = -0.5 \pm 0.3$ between 2.4 and 1 mm. From 1 mm to 3.5 μ m $\alpha = -1.06 \pm 0.02$, but the slope steepens sharply to $\alpha = -3.0 \pm 0.2$ between 3.5 and 2.2 μ m.

A possible explanation for the steep near-IR spectrum of 1413 + 135 is obscuration by dust intrinsic to the source. Two facts argue against this possibility. First, it is difficult to explain the abrupt change in spectral index at 3.5 μ m by reddening alone, as the 2.2- μ m datum lies almost 1 mag below the value extrapolated from 3.5 μ m using $\alpha = -1$. Although a detailed analysis depends on the redshift of 1413 + 135, a normal reddening law predicts a deficit of only about 0.5 mag.

Second, the 1-mm and 100- μ m data also put slight constraints on the amount of dust in the source. If the intrinsic spectrum of 1413 + 135 were characterized by $\alpha = -1.06$ into the UV, then the difference between the flux actually observed and the flux that would be observed in the absence of dust is $\sim 2.4 \times 10^{-14}$ W m⁻². If the visual-UV luminosity is absorbed and reradiated by dust surrounding the source, this flux should be observable at far-IR or millimetre wavelengths. With no knowledge of the redshift of 1413 + 135 it is impossible to place quantitative limits on the amount of dust or its temperature¹⁸. The 1-mm data correspond to a flux of 1.2×10^{-14} W m⁻² and rule out emission from very cold, redshifted dust with a total power output equal to the hypothesized deficit at visual and UV wavelengths and temperatures $T \leq 5(1+z)$ K, where z is the redshift. The 100- μ m upper limit corresponds to 9×10^{-14} W m⁻² and is not sensitive enough to preclude the possibility of emission of grains with $T \sim 30(1+z)$ K. More sensitive observations between 100 and 350 μ m would put strong constraints on the amount of dust in this source.

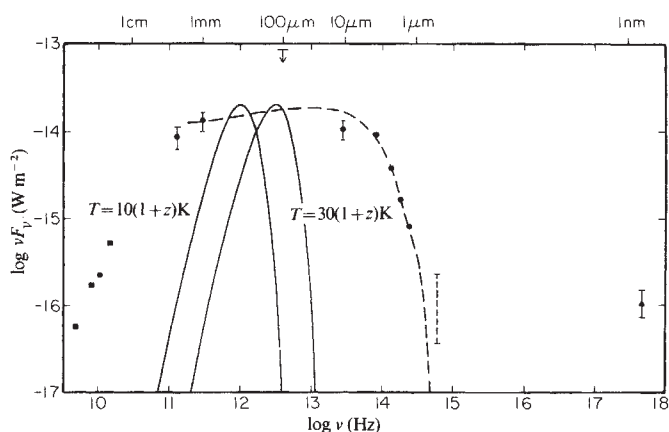


Fig. 1 The energy distribution of the source 1413 + 135 between November 1980 and April 1981. ●, New data reported in this paper (Table 1) or in ref. 4. ■, Data from Aller and Aller (personal communication). ▲, X-ray datum from ref. 14. The dashed vertical line at 0.5 μ m represents an estimate of the visual flux density of the source based on its appearance in the television guider at the 5-m telescope. The dashed curve shows a model for the optically thin synchrotron emission from 1413 + 135. The solid curves show the thermal emission from dust grains at two representative temperatures with a total flux of 2.4×10^{-14} W m⁻² and an emissivity proportional to frequency.

An alternative to attributing the redness of the energy distribution of 1413+135 to dust is to consider the synchrotron mechanism itself. Rieke *et al.*³ and Bregman *et al.*¹⁴ have suggested that the steepening of the spectrum is due to a dearth of electrons of sufficiently high energy to radiate at high frequency. The introduction of a high-energy cutoff at a Lorentz factor γ_c results in a steepening of the energy distribution at frequencies above¹⁹

$$\nu_c > 4.20 \gamma_c^2 B \text{ MHz} \quad (1)$$

where B is the magnetic field. The dashed line in Fig. 1 shows a model²⁰ for the optically thin emission with $\nu_c = 5.7 \times 10^{13}(1+z)$ Hz and $\alpha = -0.87$ at $\nu \ll \nu_c$. The visual datum lies significantly above the model and may require a second component to explain the emission at visual wavelengths; this component has been discussed elsewhere¹⁴.

The existence of a high-energy cutoff in the electron distribution rules out the possibility that the X-ray radiation from this source is due to primary synchrotron radiation. Inverse Compton scattering will, however, increase the frequency of a radio-IR photon by a factor of γ^2 . With the range of γ s observed in this object, the synchrotron-self Compton mechanism²¹ can thus explain the X-ray emission.

If the radiation from 1 mm to 1.25 μ m is caused by the incoherent electron synchrotron mechanism^{17,21}, the source must be very compact to be optically thick at wavelengths as short as 2 mm. The source size is given by²²

$$\theta = 2.2 F_\nu^{1/2} \lambda (1+z)^{1/2} T_{12}^{-1/2} \quad (2)$$

where θ is the source size in μ arc s, F_ν the flux density in Jy, λ the observed wavelength in mm, z the redshift of the object and T_{12} the brightness temperature in units of 10^{12} K. Observational studies²¹ and theoretical considerations²² suggest that the rest frame brightness temperatures for compact radio sources fall in the range 10^{11} – 10^{12} K. Using the observed 2.4-mm flux density of 7 Jy, the apparent angular radius of 1413+135 lies between $14(1+z)^{1/2}$ and $44(1+z)^{1/2}$ μ arc s for $0.1 < T_{12} < 1.0$. For a cosmology with a deceleration parameter $q_0 = +1$, the corresponding linear size of the region is given by

$$r = cz \theta H_0^{-1} (1+z)^{-2} \quad (3)$$

With a Hubble constant $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, this size is $0.4z(1+z)^{-1.5} < r < 1.3z(1+z)^{-1.5}$ pc.

Assuming the 2-keV flux density of 2.2×10^{-8} Jy from 1413+135 (ref. 14) is due to the self-Compton mechanism, it is possible to estimate the magnetic field strength, B , in the compact source, as the ratio of energy densities in the magnetic and radiation fields must be comparable with the ratio of the luminosities of the synchrotron and Compton components^{19,22}. The energy density, U_m , in the magnetic field is given by (in cgs units)

$$U_m \sim B^2/8\pi \sim (L_i/L_X)U_r \quad (4)$$

where L_i/L_X is the ratio of the total (synchrotron) luminosity to the X-ray (self-Compton) radiation. U_r is the energy density in the radiation field and is given by

$$U_r = 4\pi I/c \sim L_i/(\pi r^2 c) \quad (5)$$

Between 2 cm and 1 μ m the total luminosity is $3.7 \times 10^{40} z^2$ W (for $q_0 = +1$) where a straight line interpolation between 1 mm and 10 μ m has been assumed. The X-ray flux¹⁴ observed between 0.5 and 3.0 keV is $1.9 \times 10^{-16} \text{ W m}^{-2}$. L_X also depends on the emission outside this band. For a flat-spectrum source emitting significantly within a passband ΔE (keV) the X-ray luminosity is $8.26 \times 10^{37} z^2 (\Delta E/2.5 \text{ keV})$ W. With these values, the above equations yield for the field strength

$$B \sim 166(1+z)^{1.5} T_{12}^{1/2} (\Delta E/2.5 \text{ keV})^{-1/2} \text{ G} \quad (6)$$

leading to fields in the range 50–150 G. As noted by Rees²³, these large magnetic fields may be reduced by invoking the presence of large-scale, extremely relativistic mass motions within the object.

From the best value of ν_c and equations (1) and (6), the maximum Lorentz factor is

$$\gamma_c \sim 280(1+z)^{-1/4} T_{12}^{-1/4} (\Delta E/2.5 \text{ keV})^{1/4} \quad (7)$$

One consequence of the large magnetic fields within 1413+135 is that the half life of the emitting electrons must be quite short¹⁹.

$$t_{1/2} = 5 \times 10^8 / \gamma B^2 = 64(1+z)^{-2.75} T_{12}^{-0.75} (\Delta E/2.5 \text{ keV})^{0.75} \text{ (s)} \quad (8)$$

or a few minutes for the highest-energy electrons in the field given by equation (6). Accordingly, there must be an injection of relativistic electrons on a very rapid timescale. Bregman *et al.*¹⁴ have observed large variations in the night-to-night emission from 1413+135 at 2.2 μ m. The extreme variability around ν_c must be partly due to the exponential sensitivity of the high-frequency emission to the exact value of γ_c —a slight variation in γ_c producing a large change in the flux density.

The energy distribution of 1413+135 resembles that of a very red BL Lac object. Although it is not known whether the degree of redness of BL Lac spectra is due to differing redshifts or physical conditions within the sources, the object 1413+135 is clearly an extreme member of the class. As a far-IR extragalactic source, 1413+135 may be among the first of many sources of this type to be found in a long wavelength, all sky survey such as that planned for the IRAS satellite.

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Multifrequency observations of the red QSO 1413+135

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The extremely red QSO 1413+135 shows characteristics typical of BL Lac objects—no emission lines, is embedded in a luminous galaxy, is subject to rapid radio and IR variability, has an inverted radio spectrum and shows substantial IR polarization. The rapid steepening of the nonthermal spectrum at $\lambda < 5 \mu\text{m}$ is interpreted as synchrotron emission from an electron distribution that ends sharply at some maximum energy. The X-ray emission is probably inverse Compton radiation. We calculate that the optically thin emitting region is compact, has a large magnetic field and exhibits bulk relativistic motion towards the observer. The most energetic electrons in the emitting region have a Lorentz factor of 10^2 – 10^3 .

ATTEMPTS to determine the physical conditions of the continuum emitting regions of QSOs have failed to obtain well defined values for the model parameters because the data can be interpreted equally well by a wide variety of models. Because of its unusual spectrum, the red QSO 1413+135 proves an exception in this respect. We report here simultaneous observations of the continuous spectrum of 1413+135 from which we make the first estimate of the maximum energy of the electrons in a synchrotron source. We can also determine other parameters such as source size and magnetic field with improved accuracy.

Red QSOs^{1–3} share several properties with BL Lac objects and optically violent variable QSOs (OVVs): they are strong flat-spectrum radio emitters, variable on a time scale of weeks or less, and frequently lack emission lines. The distinguishing characteristic of red QSOs is the extremely steep decline in their continuous spectrum between IR and optical wavelengths. In terms of the energy flux distribution $F_\nu \propto \nu^{-\alpha}$, the characteristic spectral index $\alpha \approx 3$. Consequently they are readily detected in the IR, but very difficult to observe optically². The characteristic steep spectra of red QSOs is believed to be an intrinsic property of these objects, and not the result of selective extinction^{2,4}.

The red QSO 1413+135 is particularly suited to detailed study because it is one of the brightest members of its class at radio and IR wavelengths. By determining the redshift and monitoring the IR and radio flux, we have obtained essential information on the size of the emitting region. We have also obtained nearly simultaneous observations of the continuous spectrum in the radio, IR and X-ray bands. The IR observations provide a good determination of a cutoff in the spectrum. The composite spectrum permits determination of the parameters of a simple theoretical model, that is size, magnetic field and bulk velocity and the maximum energy of the synchrotron electrons. The key element in the analysis is that the unusual shape of the steeply falling IR spectrum of 1413+135 places strong constraints on theoretical models of the source of its continuous emission.

Observations

Near IR observations of 1413+135 were obtained with the 1.54-m and 2.25-m telescopes of the University of Arizona with the same IR photometers used previously. Apertures of 8.5 arc s and 7.8 arc s, with spatial chopping of 10 arc s, were used respectively with the two telescopes (calibration procedures are described elsewhere (ref. 5 and G.H.R. unpublished data)). The most extensive measurements, obtained at K (2.2 μm), provide additional information on the variability of the source (Table 1).

During January 1980 we obtained IR photometry of 1413+135 out to 10.6 μm , which are normalized to the flux level measured at 2.2 μm on 24 January 1980 (Table 2). Measure-

ments at 2.2 μm were obtained within 1 h of the measurements at the other wavelength to facilitate this normalization. The major conclusion from this photometry (Fig. 1)—that the spectrum becomes less steep beyond 5 μm —has been discussed elsewhere⁶ and confirmed by subsequent measurements⁷.

We also measured the polarization of the source at 2.2 μm on 26 January 1980 and found it to be $16 \pm 3\%$, at a position angle of $175^\circ \pm 5^\circ$ (the instrumentation is discussed in ref. 8).

Our new observations support the suggestion that the exceedingly steep spectral slope of 1413+135 is not a result of reddening of an otherwise normal quasar spectrum^{2,4,7}. Given the redshift of 1413+135, the extinction of absorbing material must be large ($A_V \approx 6$) if the IR spectrum was originally a simple power law of slope ~ 1.5 . This extinction would imply a high degree of multiple scattering at 2.2 μm , which could depolarize the source, in disagreement with observations. Furthermore, when the observed spectrum is corrected for such large absorption, it would have an optical-IR to radio luminosity ratio

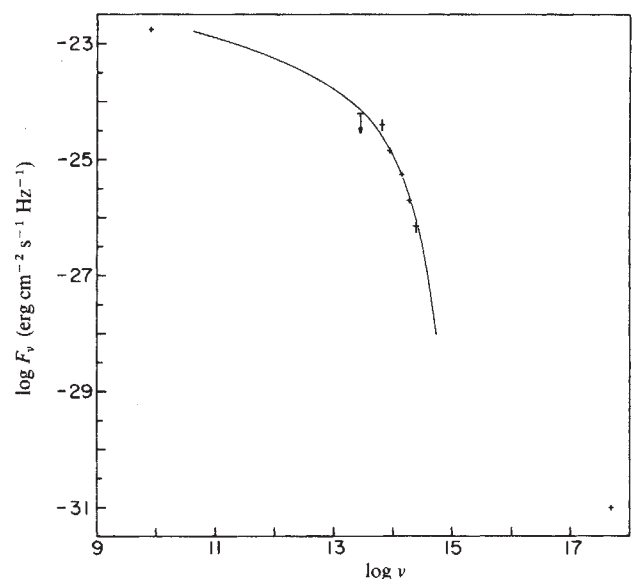


Fig. 1 Nearly simultaneous observations of 1413+135 taken during 21–24 January 1980. The data have been fitted by a theoretical spectrum of synchrotron emission from a power-law electron distribution which terminates at some maximum energy (equation (1)). The fit of the theoretical spectrum to the IR data is insensitive to the slope of the power law electron distribution (E^{-n} , $n=1.8$ here). The X-ray emission, which is clearly not an extrapolation of the IR continuum to higher energy, may be inverse Compton radiation.

Table 1 Variability of 1413+135 at 2.2 μm

Date	Flux (mJy)	Ref.
10 February 1979	3.32 $\pm$ 0.10	2
7 March 1979	1.72 $\pm$ 0.12	2
8 March 1979	1.98 $\pm$ 0.18	2
24 January 1980	5.5 $\pm$ 0.3	This work
25 January 1980	4.6 $\pm$ 0.2	This work
26 January 1980	3.7 $\pm$ 0.2	This work
10 February 1980	2.55 $\pm$ 0.2	7
27 February 1980	4.2 $\pm$ 0.2	This work
26 April 1980	2.35 $\pm$ 0.2	This work
21 May 1980	2.0 $\pm$ 0.2	This work
22 May 1980	1.8 $\pm$ 0.15	This work
23 May 1980	2.6 $\pm$ 0.2	This work
21 July 1980	1.71 $\pm$ 0.09	7
25 July 1980	2.15 $\pm$ 0.20	7
4 August 1980	1.98 $\pm$ 0.10	7
5 August 1980	1.76 $\pm$ 0.14	7

substantially larger than what is observed in all other flat spectrum radio sources⁹.

We have located 1413+135 accurately on the Palomar Sky Survey plates and confirm the original PKS position and finding chart¹⁰; the finding chart given by Condon *et al.*¹¹ is incorrect. The positions of the source measured in the radio, IR, optical and X-ray bands are identical, to within measurement error. The optical image is red and clearly extended, and we suggest that it is a distant underlying galaxy. An optical spectrum of this galaxy, obtained at the Multiple Mirror Telescope, shows the H and K break at 5,000 Å, corresponding to a redshift of $z = 0.26 \pm 0.01$ (Fig. 2). At this redshift, the next accessible strong absorption features expected in the spectrum of a giant elliptical galaxy are H δ and CH; these features are present in the data, at modest signal-to-noise ratio. There are no emission lines, and the nonthermal continuum is overwhelmed by the galaxy at $\sim 0.5 \mu\text{m}$.

X-ray observations of 1413+135 were made with the Image Proportional Counter on the Einstein Observatory as part of Guest Observer Programs 246 and 547. The first observation, which was made on 21 January 1980 (UT) with an integration time of 6,111 s, revealed a weak source coincident with the established position of 1413+135. The net counts in the 0.19–3.63 keV region (ch. 3–13) were 48.3 ± 13.9 (3.5σ). During 24 January 1981, a second observation was made with an exposure time of 11,954 s. The net counts in the 0.19–3.63 keV region were 148 ± 18.6 (8σ). It is not possible to determine whether the difference in the counting rates between these two observations is significant (0.0079 ± 0.0023 c.p.s. (counts per s) for 1/80 and 0.0124 ± 0.0016 c.p.s. for 1/81, a 1.6σ difference).

To convert the counting rate to fluxes, we used a column density for neutral hydrogen of $1.4 \times 10^{20} \text{ cm}^{-2}$ (estimated from ref. 12) and a range of (unknown) X-ray spectral indices of 0.75, 1.5 and 3.0. Average values for the integrated fluxes (0.19–3.63 keV) and monochromatic fluxes (2 keV) are $1.59 \times 10^{-13} \text{ erg cm}^{-2} \text{ s}^{-1}$ (1/80), $2.50 \times 10^{-13} \text{ erg cm}^{-2} \text{ s}^{-1}$ (1/81) and $1.47 \times 10^{-5} \text{ keV cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$ (1/80), $2.31 \times 10^{-5} \text{ keV cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$ (20% systematic uncertainty in the conversion to fluxes).

The peculiar IR–optical spectrum makes it difficult to compare the X-ray brightness of this object with that of other QSOs. While the ratio of X-ray to optical flux ($\alpha_{\text{ox}} = 1.3$) is typical of BL Lacs or QSOs (refs 13, 14 and W.H.–M. Ku, personal communication), the X-ray to IR flux ratio ($\alpha_{\text{ix}} = 1.6$) is larger than the mean of radio bright QSOs¹³.

The flux density and linear polarization of 1413+135 were observed at 4.8, 8.0 and 14.5 GHz using the University of Michigan 26-m paraboloid (instrumentation and reduction described elsewhere^{15,16}). These flux density measurements were corrected for source polarization and are on a flux density scale in which 3C274 has assumed fluxes of 70.0, 48.6 and 29.9 Jy at 4.8, 8.0 and 14.5 GHz respectively.

Figure 2 clearly illustrates that the radio spectrum was inverted during the entire observing period. Between June and August 1980, the source increased its flux at 14.5 GHz by 100%, giving us a time scale for flux variation (Fdt/dF) of about 3 months. As the source became brighter, the spectrum became more steeply inverted; α ($F_\nu \propto \nu^{-\alpha}$) decreased from -0.2 to -0.9 . A second outburst, which occurred from January to April 1981, has properties similar to those of the first outburst: the flux changes occur simultaneously at all three frequencies but the intensity of the outburst is greatest at the highest frequency (14.5 GHz).

Both the lack of any detectable time delay between the peak at 14.5 and 8.0 GHz and the relative amplitude of the outburst at these frequencies are inconsistent with the simple expanding source model¹⁷. The ratio of the amplitude of the outburst at 14.5 GHz compared with that at 8.0 GHz is considerably greater than most outbursts in other sources. The spectral slope ($\alpha = -d \log F/d \log \nu$) of the flux increase (first outburst) is $\alpha = -2.5 \pm 0.3$; the second outburst has a similar value of α . Because a homogeneous optically thick synchrotron source has a spectral index of -2.5 , these outbursts may be caused by newly injected or newly shocked plasma that is optically thin at IR frequencies and becomes optically thick somewhere in the far IR to millimetre region. If the cooling time of synchrotron-emitting electrons equals the decay time of the outburst, $B \geq 1$ G, which is consistent with the analysis of the simultaneous spectrum.

In contrast to the large IR polarization, we have not detected any linear polarization in the source at radio wavelengths. Averages of the 1980 data yielded degrees of polarization of 0.5 ± 0.3 , 0.4 ± 0.5 and $1.4 \pm 0.7\%$ at 4.8, 8.0 and 14.5 GHz respectively.

Discussion and interpretation

The above observations strengthen the association of 1413+135 with the BL Lac objects. In addition to the absence of emission lines (this work and ref. 7), the IR flux variations (Table 1) place 1413+135 among the most variable extragalactic sources known. On two occasions (late January 1980 and late May 1980), changes $>20\%$ were observed on time scales of 1 day and, on three occasions, the intensity changed by a factor of two in a month or less (Table 1). Among well studied sources, none show a significantly higher level of IR activity, and only a few (for example, BL Lac, OI 090.4) display comparable variability (see ref. 18 and refs therein). Similarly, 1413+135 is one of the most rapidly variable radio sources, exhibiting a 100% flux increase at 14.5 GHz in a little less than 3 months. The high level of IR polarization also supports the suggestion² that the source is a BL Lac object. Only one-third of the BL Lac objects listed by Angel and Stockman¹⁸ have larger polarizations than that of 1413+135. Its low degree of radio polarization is not

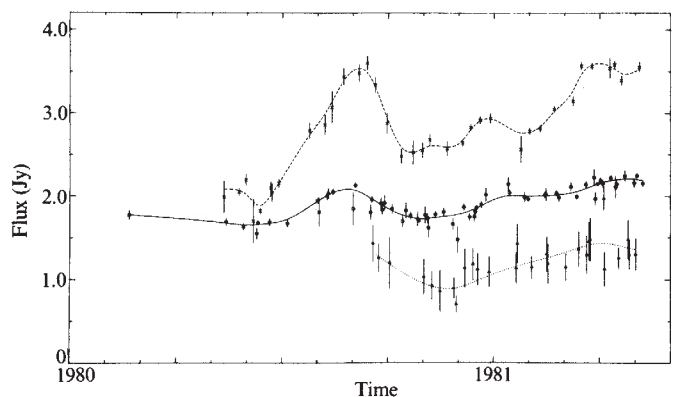


Fig. 2 The radio observations of 1413+135 show that this source is extremely active at 14.5 GHz (x) and less active at 8 GHz (o) and 4.8 GHz (Δ). The radio spectrum, which is always inverted, becomes most steeply inverted during outbursts ($\alpha = 0.9$). The rate of flux increase during the June–August 1980 and February–April 1981 outburst are nearly identical.

Table 2 IR spectrum of 1413+135 on 24 January 1980

Wavelength (μm)	Flux* (mJy)
1.25	0.76 ± 0.11
1.6	1.92 ± 0.17
2.2	5.48
3.5	14.5 ± 1.2
4.6	39 ± 10
10.6	≤ 63 (2σ)

* Flux values have been corrected for filter bandpass effects to the equivalent monochromatic flux at the indicated wavelength.

unusual for BL Lac-type objects, although several BL Lac objects display high radio polarization. Another similarity of 1413+135 with the BL Lac objects is that it probably lies in the centre of an elliptical galaxy ($z=0.26$). A study of the environment in which red QSOs, like 1413+135, exist is aided by the exceptionally steep nonthermal spectrum, which does not interfere with the identification of galactic spectral features.

One of the most striking features of the composite nonthermal spectrum (Fig. 1) is that the X-ray data cannot be explained as an extrapolation of the IR data to higher frequencies; any smoothly extrapolated curve falls orders of magnitude below the observed X-ray flux. The radio-optical continuum is usually interpreted as synchrotron radiation produced by relativistic electrons. High polarization at $2 \mu\text{m}$ is consistent with this interpretation. We suggest that the X-ray emission, which is clearly not an extension of the radio optical emission, is inverse Compton radiation.

The abrupt steepening in the spectrum near $5 \mu\text{m}$ from $\alpha \approx 0.8$ (ref. 4) to $\alpha \approx 3$ cannot be achieved by any evolutionary model¹⁹. The sharpest spectral cutoff occurs for an electron distribution which ends abruptly at some energy, such as

$$\begin{aligned} N_e &= N_0 \gamma^{-n} & \gamma < \gamma_2 \\ N_e &= 0 & \gamma > \gamma_2 \end{aligned} \quad (1)$$

The general solution for the spectrum emitted by this electron distribution²⁰ has been solved here for several values of n (equation (1)). These solutions describe a simple power law of slope α for frequencies below a critical frequency ν_2 ($\alpha = (n-1)/2$ in this region), while the spectrum steepens very sharply above ν_2 , eventually becoming exponential for $\nu \gg \nu_2$ ($\nu^{0.5} \exp(-\nu/\nu_2)$). An important feature of these solutions is that their shape is insensitive to the value of n (or α) at high frequencies ($\nu > \nu_2$). As demonstrated in Fig. 1, the solutions fit the IR data extremely well. The range of critical frequencies ν_2 , which are determined for each fit (Table 3) corresponds to the wavelength range $2.6\text{--}3.8 \mu\text{m}$ ($\alpha = 0.3\text{--}1.5$).

The sharpness of the spectral cutoff forces us to make two strong qualitative conclusions. First, the electron distribution in the emitting region terminates abruptly at some energy. Second, the magnetic field is fairly uniform in the IR emitting region. More precisely, magnetic fields above some critical field strength either do not exist, or they occupy an insignificant volume in the emitting region in which the IR photons are produced. Furthermore, if the relativistic electrons retain their velocity distribution between the emitting region and the region in which they were created, then the electron acceleration process is not diffusive in energy.

The size of the emitting region, its magnetic field, as well as the energy cutoff corresponding to ν_2 can be determined within the

framework of a theoretical model. We have chosen the homogeneous synchrotron-self-Compton model of Jones *et al.*^{21,22} with the added feature that the emitting region may have a bulk velocity towards the observer in the quasar rest frame (that is, a special case of relativistic motion is allowed). Equations (33) and (37) of Jones *et al.*²² were solved, with various correction factors set to unity (that is $(\epsilon_{sc} G_{sc})^{0.5} f^{-\alpha-1} = 1$). To apply this model, one must know the frequency and flux at which the IR emitting region becomes optically thick (ν_b and F_b) as well as the shape of the electron distribution (n or α). Only two of these quantities are independent.

Our simultaneous observations are consistent only with the models for which $1.5 \geq \alpha \geq 0.3$. If non-simultaneous observations⁴ can be used to estimate α and the turnover frequency, $\alpha \approx 0.9$, and $\nu_b = 1.6 \times 10^{11} \text{ Hz}$ (this defines a preferred model, no. 3). To obtain model parameters, we fit the calculated spectrum to the IR spectrum, and for each value of α , we find a value for F_b consistent with our spectrum or that of Beichman *et al.*⁴. The model equations are solved assuming that the X rays arise from the inverse Compton process, and with the condition that the size determined from temporal flux variation equals the size determined with the synchrotron-self-Compton model.

The temporal flux variations of 24–26 January 1980 at $2.2 \mu\text{m}$ place an upper limit on the size of the emitting region (for an optically thin emitting region, $\beta \approx 2$):

$$s \leq \beta c |F_\nu| dt/dF_\nu / (1+z') = 8 \times 10^{-3} / (1+z') \text{ pc} \quad (2)$$

where z' includes both cosmological expansion plus the motion of the emitting region with respect to the quasar rest frame. The model solutions are presented in Table 3, where $B \sin \theta_0$ is the magnetic field (θ_0 is the mean angle the magnetic field makes with our line-of-sight), d is the radius of the emitting region, θ is

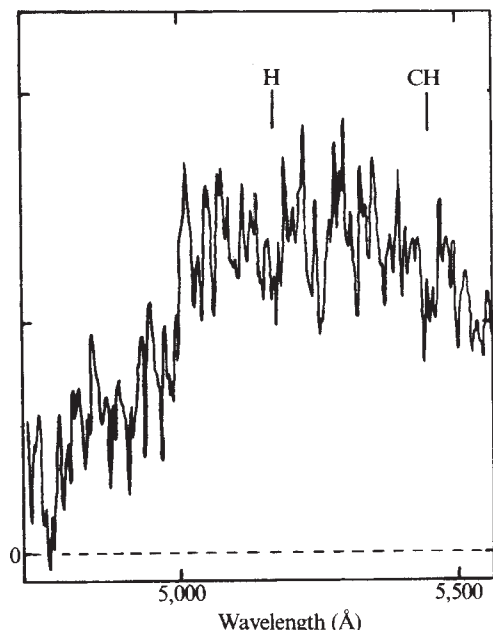


Fig. 3 The clear break at $5,000 \text{ \AA}$ in the galaxy around 1413+135 is interpreted as the H and K break redshifted by $z=0.26$. The $H\delta$ and CH absorption features, denoted here by H and CH, appear weakly in this spectrum. The spectral resolution is $\sim 10 \text{ \AA}$, and the observations were made through a 2.5 arc sec round aperture.

Table 3 Theoretical model results

Model no.	α	ν_2 (Hz)	ν_b (Hz)	F_b (Jy)	θ (ms)	$B \sin \theta_0$ (G)	r (pc)	γ_2	Γ
1	0.4	$7.8+13$	$2.2+10$	2.0	1.1–1	$8.2+0$	$5.0-2$	$6.1+2$	4.0
2	0.6	$7.9+13$	$6.6+10$	4.0	6.7–2	$3.2+1$	$3.9-2$	$3.4+2$	3.2
3	0.9	$8.5+13$	$1.6+11$	7.0	4.3–2	$6.1+1$	$3.1-2$	$2.9+2$	2.6
4	0.9	$8.5+13$	$1.4+12$	1.0	4.6–3	$6.4+3$	$1.0-2$	$5.0+1$	1.03
5	1.5	$11.5+13$	$1.8+12$	5.0	6.9–3	$2.3+3$	$1.3-2$	$8.8+1$	1.24

the angular size of the optically thin region one would observe, γ_2 is the cutoff energy of the electron spectrum, and Γ is the Lorentz factor which describes the motion of the emitting region with respect to the quasar rest frame. Because temporal flux variations may yield only a lower limit to the source size, we formally determine upper limits to $B \sin \theta_0$, θ and r , and lower limits for γ_2 and Γ . However, in the following discussion, we assume that the size determined from IR flux variations is a good measure of the size of the emitting region (that is equation (2) is an equality).

Several of these models may be ruled out because the results violate fundamental physical considerations. The electrons must be energetic enough to scatter synchrotron photons to X-ray energies; this places the limit $\gamma_2 > 10^2$. Therefore, all models with $\alpha > 1.3$ or $\nu_b > 10^{12}$ must be eliminated from consideration. The difference in the values of B , r , γ_2 and Γ between the preferred model (no. 3) and the models allowed by our data (nos 1, 2) is not large. Note that this is the first time that γ_2 (~ 300) has been calculated.

The derived values for the magnetic field (60 G) and the source size (~ 0.03 pc) differ significantly from the canonical values derived for other sources (typically several microgauss and 0.1–100 pc (ref. 22)). An independent argument in support of this field strength is that if the IR flux variation is attributable to electrons losing their energy in the time defined by temporal flux variation, then $B \sin \theta_0 > 0.2$ G.

The finding that the emitting region is moving away from the quasar towards us at relativistic velocities is consistent with either a beam model or an expanding shell model for the emitting region. The value of Γ we derive (≈ 3) is similar to what other investigators estimate for BL Lac objects^{16,22}.

Beichman *et al.*⁴, who used a model which does not allow for relativistic motion and does not use the X-ray flux as a measure of the inverse Compton radiation, obtain results similar to our own.

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Conclusions

We have studied the radio, IR, optical and X-ray spectrum of the extremely red QSO 1413 + 135 and find: (1) It is embedded in a luminous galaxy at a redshift of $z = 0.26 \pm 0.01$. (2) It is very closely related to BL Lac-type sources, as shown by its pattern of variability in the radio and IR, by its strong IR polarization and by its lack of emission lines. (3) The radio spectrum, which is always inverted, is subject to large outbursts that occur over a period of a few months. While these outbursts occur simultaneously at 8 and 14.5 GHz, the greatest amplitude occurs at 14.5 GHz. The observations exclude the simple expanding source model. (4) Its nonthermal spectrum steepens abruptly near 5 μm , requiring that the electron energy distribution terminate sharply at some maximum energy (Lorentz factor 10^2 – 10^3) and that the magnetic field is uniform within the IR emitting region. (5) The X-ray data lie far above an extrapolation of the IR spectrum to high frequencies. We suggest that the X-ray emission is produced by the inverse Compton process. (6) The magnetic field in the source is probably > 1 G, and the emitting region (~ 0.03 pc) must be moving towards us with a Lorentz factor between 1.5 and 4.

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Expression of a human gene for interferon in yeast

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A DNA sequence coding for mature human leukocyte interferon D (LeIF-D) was linked with DNA fragments of the 5'-flanking sequences of the Saccharomyces cerevisiae (yeast) alcohol dehydrogenase I gene in a plasmid capable of autonomous replication and selection in both yeast and Escherichia coli. Yeast cells transformed by these plasmids synthesize up to 1×10^6 molecules of biologically active LeIF-D per cell.

In addition to its usefulness as a model for studying eukaryotic cellular processes, the yeast *Saccharomyces cerevisiae* is a suitable host cell in gene expression experiments. The yeast transformation procedure^{1,2} allows both the reintroduction of cloned and *in vitro* mutated yeast DNA sequences (to map functional sites) and the direct cloning of yeast genes by functional

complementation of defective mutants³ (for review see ref. 4).

Similarly, a particular genomic sequence from another eukaryotic organism might be isolated by complementation of defined yeast mutants affecting genes common to both organisms. While this approach has been successful for a *Drosophila* gene corresponding to the yeast ADE8 locus,

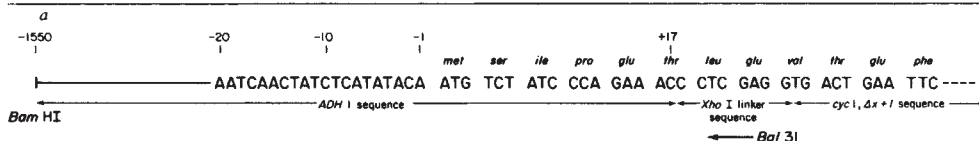
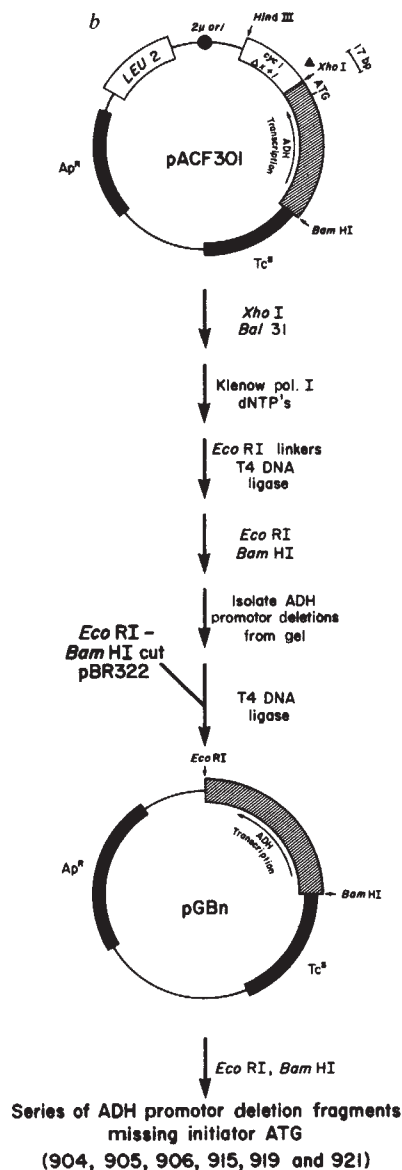


Fig. 1 *a*, DNA sequence of the *ADH1*-*CYC1* gene fusion in pACF301. The promoter and first 10 bp of the *ADH1* structural gene and the structural gene of *cyc1* Δx+1 are joined using an *Xho*I linker. *b*, Construction of yeast *ADH1* promoter fragments. Ten μg of *Xho*I-cut pACF301 were incubated with 0.2 U of *Bal*31 nuclease (BRL) for 15–30 s at 30 °C in 20 mM Tris pH 8.1, 12 mM CaCl₂, 12 mM MgCl₂, 0.2 M NaCl, 1 mM EDTA and 0.1 μg ml⁻¹ bovine serum albumin (BSA). During this time ~30–70 bp of the DNA were removed. A 1-μg aliquot of this DNA was incubated with DNA polymerase I (Klenow fragment) and deoxynucleoside triphosphates to fill in the ends, and then incubated with T4 DNA ligase and synthetic *Eco*RI linkers (Collaborative Research) for 12 h at 14 °C. The DNA was then cleaved with *Eco*RI and *Bam*HI restriction nucleases and the mixture of fragments containing variously deleted *ADH1* promoter sequences isolated by preparative electrophoresis on 1% agarose gel. The mixture of electroeluted fragment was ligated with the large *Eco*RI-*Bam*HI fragment of pBR322, and individual clones selected by transformation of *E. coli* strain RRI, selecting for Ap^R colonies. DNA from individual colonies was prepared by a quick-screening procedure²⁶. The length of each deletion was estimated by cleaving each plasmid with *Eco*RI endonuclease, 3'-end labelling with Klenow polymerase plus [α-³²P]dATP, then cleaving the DNA with endonuclease *Alu*I and sizing the resulting small fragments (containing the deleted part of the *ADH1* leader region) on a urea-acrylamide gel.



that the promoter region of the yeast *ADH1* gene was able to confer its transcription starting specificity to the LeIF-D coding-region DNA.

The yeast *ADH1* promoter

The DNA sequence has been determined for the yeast *ADH1* gene, including the 750 base pairs (bp) which flank the 5' end of the protein-coding region. The 5' termini of *ADH1* mRNA molecules made in yeast map at two positions, 27 and 37 bp upstream from the translation start⁸. The DNA sequences which specify the position of the 5' ends (presumably the transcript starts) of these mRNA molecules are not contained within the *ADH1* coding region but instead lie within the upstream DNA (G.A. and B.H., unpublished results). Fused genes containing the 5'-flanking and N-terminal coding sequences of the *ADH1* gene and coding portions of *CYC1* gene⁹ retain the *in vivo* transcription starting specificity of *ADH1*. The plasmid ACF301 (Fig. 1a,b) which contains *ADH1* gene sequences extending from -1,550 through the ATG initiation codon to a point 17 bp into the coding region is an example of such a fusion. This *ADH1* fragment was joined, via a synthetic *Xho*I DNA linker, to a *CYC1* structural gene (*cyc1* Δx+1) in which the A of initiator ATG had been deleted. When pACF301 was introduced into yeast cells, the *in vivo* transcripts of the *cyc1* Δx+1 gene had 5' termini at the same positions in the *ADH1* flanking sequence (-27 and -37) as the 5' ends of normal mRNA from the chromosomal *ADH1* gene. These 5' transcript ends contrast with those transcribed from the *CYC1* gene attached to its original *CYC1* 5'-flanking region; they are distributed amongst seven start sites which are further removed from the *CYC1* coding region¹⁰.

The above evidence that transcript starting specificity may be transferred from one yeast gene to another prompted the use of the *ADH1* promoter for heterologous expression—to promote mRNA synthesis from a human gene in a yeast cell. To facilitate attachment of the LeIF-D coding sequence, the *ADH1* promoter sequence derived from pACF301 was treated with exonuclease *Bal*31 to delete the small amount of *ADH1* coding sequence it contains (Fig. 1b). After attaching *Eco*RI linkers, the resulting set of *Eco*RI-ended *ADH1* promoter fragments (Fig. 2) have their right-hand (3') end points at six different positions within the region specifying the *ADH1* untranslated leader. By fusing each one of these with the LeIF-D coding region and transforming yeast cells with the resulting recombinant plasmid, it was possible to test the effect of varying leader lengths and varying cap sequence and AUG sequence contexts on functional expression of the interferon gene in yeast.

The LeIF-D gene

The human LeIF gene family consists of at least 12 distinct intronless genes^{7,11,12}. Each gene codes for a hydrophobic 23-amino acid signal precursor peptide followed by a mature polypeptide of 165 or 166 amino acids. Several features of the LeIF-D gene sequence⁷ make it suitable for testing human gene expression in yeast. (1) Because the gene does not normally contain introns, its mRNA sequence and physical conformation allow it to be transported from nucleus to cytoplasm without any need for RNA splicing. (2) The DNA sequence of the LeIF-D coding sequence indicates a biased usage of the codons in the genetic dictionary which resembles that of genes for abundant yeast proteins⁸. In particular, the arginine codons in LeIF-D are all AGA or AGG (AGA is the preferred arginine codon in yeast). (3) Interferon synthesis in yeast can easily be measured using sensitive *in vitro* antiviral assays.

The LeIF-D cDNA clone used for these experiments was isolated from a cDNA library prepared from a myeloblastoid

Drosophila genes complementing mutants at other yeast loci have not been obtained⁵, suggesting that one or more of the signals for gene expression may frequently differ between yeast and higher eukaryotes. Further evidence for such differences in functional sites came from studies on the *in vivo* transcription of a rabbit globin gene introduced into *S. cerevisiae* on a yeast plasmid vector⁶. Transcription appeared to start at a location downstream from the normal initiation point and to terminate prematurely within the second intron of the β-globin gene. Removal of the first intervening sequence by RNA processing did not occur in this aberrant transcript.

To maximize the chances for functional expression of higher eukaryotic DNA in yeast, we have replaced the 5'-promoter-leader region of the human leukocyte interferon D (LeIF-D) gene⁷ with that from the yeast *ADH1* gene. We have inserted these fused transcription units into a replicating plasmid vector and tested their ability to function in yeast. Full-length, biologically active interferon molecules were produced, indicating

Table 1 Interferon activity in yeast extracts

Orienta- tion	ADH promoter fragment	Plasmid	Units per ml of extract	Units per l of cells ($\times 10^{-6}$)	Units per l of cells $A = 1 \times 10^{-6} \dagger$	Cells per l of culture $\ddagger$ ($\times 10^{-10}$)	Per cent of cells with plasmid $\ddagger$	Units per cell containing plasmid ($\times 10^4$)	Molecules per cell containing plasmid $\S$
I	904	pFRS3	47,000	1.4	1.2	2.1	19	3.5	53,000
I	905	pFRS7	47,000	1.4	0.54	3.4	30	1.4	21,000
I	906	pFRS12	125,000	3.8	1.8	2.7	19	7.4	110,000
I	906	pFRS36	187,500	5.6	2.3	3.0	16	12	180,000
I	915	pFRS23	125,000	2.1	0.81	3.6	21	2.8	42,000
I	921	pFRS35	250,000	7.5	2.8	4.5	18	9.2	140,000
I	919	pFRS34	93,750	2.8	1.4	2.2	17	7.6	110,000
II	904	pFRS2	<1,900				22		
II	905	pFRS6	<1,900				23		
II	906	pFRS17	<1,900				36		
II	906	pFRS11	<1,900				13		
II	915	pFRS22	<1,900				46		
II	921	pFRS26	<1,900				25		
II	919	pFRS33	<1,900				31		

* Yeast cells were grown in 5 ml of YNB+CAA [0.67% yeast nitrogen base, 0.5% casamino acids and 2% glucose (Trp^+ selection)] at 30 °C to an absorbance (A) of 1.2–2.8 at 660 nm and spheroplasted with zymolyase²⁵. The final pellet was resuspended in 0.15 ml of 7 M guanidine HCl and 1 mM phenylmethylsulphonyl fluoride. The extracts were assayed using 1:100 or 1:1,000 dilutions in 150 mM NaCl, 20 mM sodium phosphate (pH=7.9), 0.5% bovine serum albumin using a vesicular stomatitis virus challenge of MDBK (bovine kidney) tissue culture cells¹⁵.

$\dagger$ Units per l of cells at $A_{660} = 1 \times 10^{-6}$ is a normalization because different cultures were collected between 1.2 and 2.8 A_{660} .

$\ddagger$ The number of yeast cells per culture was determined by dilution and plating on YNB+CAA+tryptophan (50 $\mu\text{g ml}^{-1}$) plates. To find the percentage of cells having plasmid (Trp^+ complementation), cultures were also plated on YNB+CAA (without tryptophan).

$\S$ Molecules per cell containing plasmid was calculated assuming that purified leukocyte interferon D has a specific activity of 2×10^8 U mg^{-1} and a molecular weight of 20,000.

cell line⁷. The DNA sequence of the cloned LeIF-D cDNA is nearly identical with that of the IFN- $\alpha 1$ gene described by Nagata *et al.*¹¹ and has been directly expressed in *Escherichia coli* to yield about 20,000 molecules of biologically active, mature LeIF-D per cell (P. Gray and D.G., unpublished results). The construction of the LeIF-D coding region used in these experiments closely paralleled that used for production of LeIF-A in *E. coli*¹³. Briefly, the DNA encoding the 23-amino acid signal peptide was replaced by an ATG translational initiation codon preceded by an *EcoRI* sticky end. There is also a naturally occurring *EcoRI* site present 60 nucleotides 3' of the termination codon for LeIF-D. Therefore, the resulting 560-bp *EcoRI* fragment of LeIF-D can easily be inserted into expression vectors in which the RNA polymerase II promoter and termination elements flank an *EcoRI* site.

Construction of fused ADH1-LeIF-D transcription units

Plasmid YRp7 (refs 14, 17, 28) combines the desirable features of an *E. coli* replication origin, a gene (Ap^R) allowing selection of *E. coli* transformants, a yeast replication origin (*ars1*)¹⁴ and a yeast genetic marker (*TRP1*), permitting selection of yeast colonies containing cells transformed by this plasmid. The unfortunate presence of two *EcoRI* sites in YRp7 was eliminated by removing the *EcoRI* site between *ars1* and Ap^R gene. Figure 3 shows the details of this construction and the resulting vector, pFRL4.

The *BamHI-EcoRI* fragment (~1.5 kbp) containing each of the six different yeast *ADH1* promoter fragments was ligated to

BamHI-EcoRI-cut pFRL4 DNA to give a series of expression plasmids (pFRPn). Each of the plasmids was cleaved at its single *EcoRI* site, the 560-bp LeIF-D *EcoRI* fragment was inserted and the resulting plasmids used to transform *E. coli* (Fig. 3). In the resulting recombinant plasmid clones, the various elements are joined together in the order *BamHI-ADH* promoter ($5' \rightarrow 3'$)-LeIF-D-*TRP1* ($5' \rightarrow 3'$)-*ars1*- Ap^R . Because the *ADH1* promoter and *TRP1* gene are aligned in the correct orientation, *ADH1*-promoted transcripts which read throughout the LeIF-D sequence can continue through the *TRP1* gene and be terminated at its 3' end. Hybridization studies of the *in vivo* transcripts produced from these fusions (see below) suggest that sequences for polyadenylation and/or transcription termination occur distally to the *TRP1* coding region.

Among the clones resulting from this ligation of LeIF-D to the yeast expression vectors, both orientations of the interferon sequence were obtained for all six promoter fragments. In one case, two LeIF-D coding sequences were inserted in tandem, in parallel orientation to *ADH1* and *TRP1*. *E. coli* transformants carrying each of the *ADH1*-LeIF-D fusion plasmids were grown in sufficient quantity to prepare cell extracts and to test for interferon production, using the cytopathic effect inhibition assay¹⁵ which measures interferon levels by inhibition of viral lysis of tissue culture cells. No interferon was detected in any of the *E. coli* extracts, suggesting that the yeast *ADH1* flanking sequence is unable to provide either an effective promoter for *E. coli* RNA polymerase, a binding site for *E. coli* ribosomes, or both.

Transformation of yeast and interferon expression

Recombinant plasmids, in which the LeIF-D gene was placed either in parallel or antiparallel orientation between each of the *ADH* promoter fragments for the *TRP1* gene, were introduced into yeast, using standard transformation procedures^{1,2}. Spheroplasts of the yeast recipient strain RH218 (*trp1*)¹⁶ were separately incubated with the DNA of each plasmid and the mixture then placed in agar without tryptophan to select transformant colonies. Cultures of each transformant were grown using selective pressure in liquid medium without tryptophan, collected in mid-log phase and used to prepare cell extracts by spheroplasting and guanidine hydrochloride lysis. A substantial level of interferon was observed in all transformants with plasmids having the LeIF-D gene in the correct orientation

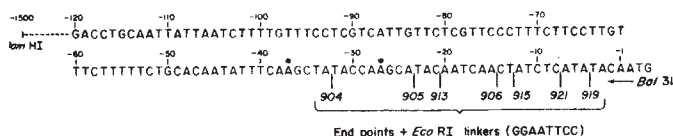


Fig. 2 *ADH1* promoter fragments used in expression experiments. The *BamHI-EcoRI* promoter fragments, isolated as described in Fig. 1b, are shown as part of the *ADH1* DNA sequence given from -120 to the initiation codon. The various promoter fragments are designated 904, 905, 906, 915, 921 and 919. The lines above the numbers show where the *ADH1* sequence ends and the *EcoRI* linker begins. The DNA sequence at the right end of the fragments was initially estimated as described in Fig. 1 legend. Later the exact sequence was determined using the pFRPn plasmid series in Fig. 3. The plasmids were cut with *EcoRI*, ^{32}P -labelled with [γ - ^{32}P]ATP using T4 polynucleotide kinase (NEN), cut with *AclI* (-190), the *AclI-EcoRI* fragment isolated by electroelution and sequenced through the linker using the Maxam and Gilbert procedure²⁷. * Transcription starts.

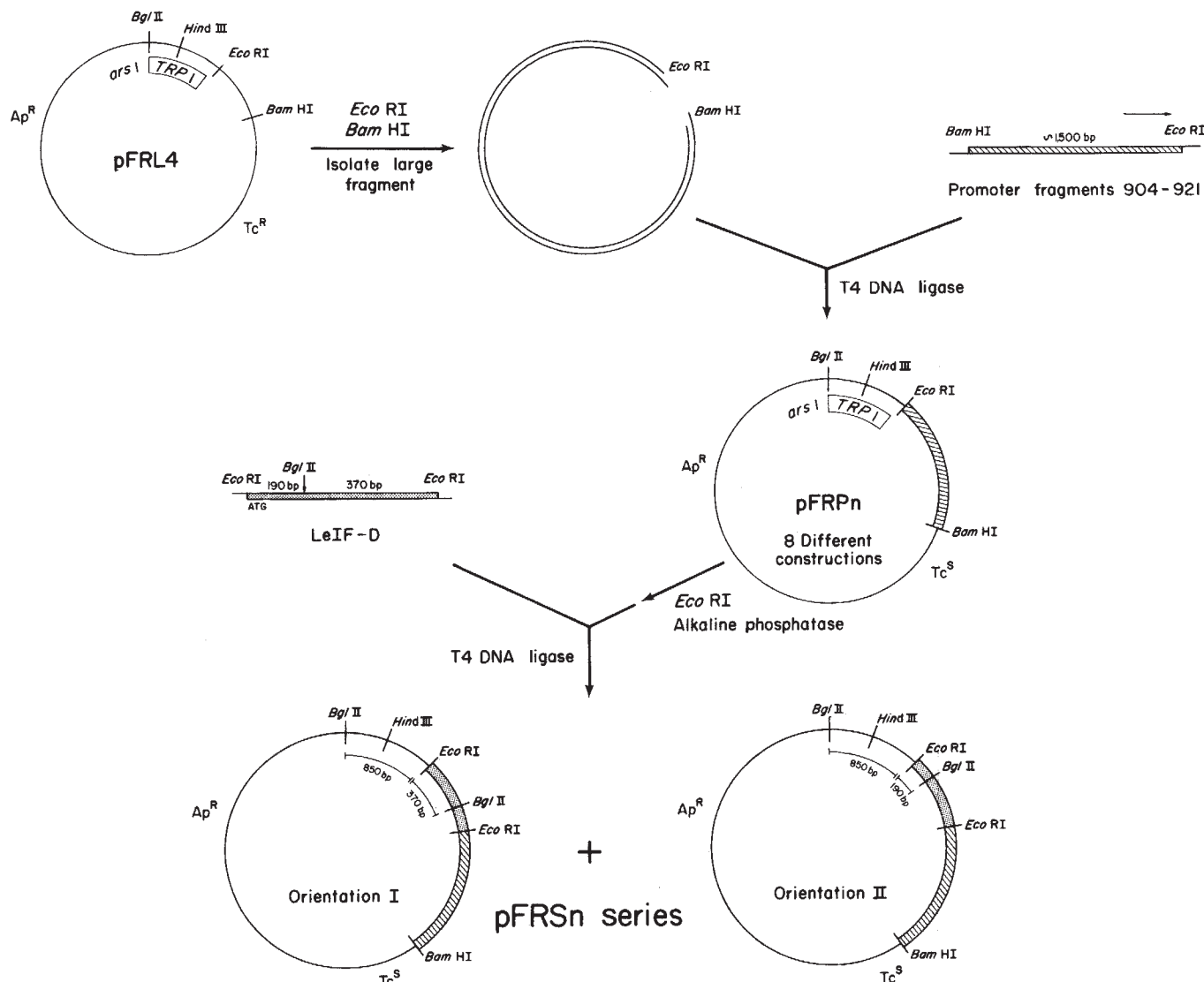


Fig. 3 Construction of expression plasmids for LeIF-D. pFRL4 was constructed from YRp7 (refs 14, 17, 28) by removing both *Eco*RI sites (Klenow DNA polymerase I fill-in of sticky ends followed by blunt-end ligation) and replacement of one *Eco*RI site using the small *Hind*III fragment of YRp7 to replace the small *Hind*III fragment of the plasmid lacking both *Eco*RI sites. Twenty μ g of pFRL4 were digested with *Bam*HI and *Eco*RI, then electrophoresed on a 1% agarose gel. The large (~5 kb) fragment was cut from the gel, electroeluted, extracted twice with phenol and chloroform before ethanol precipitation. Three μ g of this fragment were then separately ligated with 1 μ g of each of the promoter-containing fragments for 12 h at 15 °C in 50 μ l of 20 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 10 mM dithiothreitol, 0.5 mM ATP, containing 0.5 U of T4 DNA ligase. *E. coli* K-12 strain 294 was transformed with the ligation mix to ampicillin resistance and plasmids from each of these different transformation mixtures were purified (pFRPn series). Fifty μ g of pLeIF-D trp1 (P. Gray, D.G., unpublished results) were digested with *Eco*RI, then electrophoresed on a 1.2% agarose gel. The 560-bp leukocyte interferon D gene fragment was cut from the gel, electroeluted and extracted twice with phenol-chloroform before ethanol precipitation. The interferon gene was then ligated into the unique *Eco*RI site in the pFRPn plasmids which had been previously cut with *Eco*RI and treated with bacterial alkaline phosphatase. The plasmids were then analysed by *Bgl*II restriction analysis and used for yeast transformations. Both orientations of the interferon gene containing fragment were obtained in the *Eco*RI site of the pFRPn plasmids. Orientation I plasmids, with the interferon gene in line with the *ADH1* promoter fragments, gave a 1.2-kbp fragment after *Bgl*II cutting; orientation II gave a 1.0-kbp fragment. Arrows show direction of transcription.

(Table 1); the values ranged from 47,000 to 250,000 units of activity per ml of extract or from 0.5 to 2.8×10^6 units per litre of culture at A_{660} of 1. On the other hand, yeast transformants bearing fusion plasmids with the reverse orientation of the LeIF-D sequence had the same background level as yeast strains carrying no plasmid.

To assess the significance of the bulk levels of interferon observed in yeast extracts, it is necessary to measure and correct for the extent of loss of the recombinant plasmid during growth. Our relevant data (Table 1) agree with previous studies of YRp7 instability¹⁴ which show that even when *TRP1* is selected for, a high proportion of cells have lost the plasmid. If all the interferon measured in these yeast cell extracts comes from the 16–30% of the cells retaining the *Trp*⁺ character, then the actual number of interferon molecules produced may be as high as 180,000 per yeast cell.

Detailed and repetitive measurements of the interferon level made for the two expression plasmids pFRS35 and pFRS36

show, in different conditions of cell growth and extract preparation, interferon levels five- to sixfold higher than those in Table 1 (or $\sim 10^6$ molecules per plasmid-containing cell). These high values are obtained with growth of moderate-sized cultures in shake flasks, guanidine hydrochloride being removed from the extract by gradual dialysis, and allow estimation of the level of interferon production at $\sim 0.4\%$ of total cell protein. Again correcting for plasmid instability, individual yeast cells may produce as much as 1–2% of their protein as LeIF-D. In addition, preliminary experiments with stable yeast transformants containing *ADH1*-LeIF-D fusions in a yeast 2 μ plasmid vector have produced interferon levels of this magnitude in the entire culture of yeast cells.

Furthermore, as the leader peptide sequence, thought to be associated with the secretion process in specialized human cells, is not present as part of this LeIF-D structural gene, one would not expect secretion into the media during growth of the yeast. Indeed, no LeIF-D activity was found in the media from these

yeasts. Also only about 1% of the interferon was present in the supernatant after spheroplasting, suggesting that the interferon is not associated with the cell wall; it thus appears to be associated with the cytosol.

The nature of interferon produced in yeast

In all the *ADH1* leader-promoter deletions we have made, the ATG initiator triplet was removed. Provided the 5' ends of mRNAs transcribed in yeast from the *ADH1*-LeIF-D fusions occur at the *ADH1* mRNA 5' ends, the first AUG in each fusion mRNA will correspond to the synthetically added ATG. Therefore we would expect to find LeIF-D synthesized in yeast to be the same size as that produced in *E. coli*. Thus, after SDS-polyacrylamide gel electrophoresis (PAGE) of extracts of LeIF-D-producing *E. coli* and yeast, two lanes containing yeast extract compared with *E. coli* extract were simultaneously sliced, eluted and assayed to compare sizes of the peptides. Figure 4A shows both to have a molecular weight of ~20,000, the size expected for LeIF-D. The LeIF-D synthesized in yeast (using *ADH1* promoter fragment 906) has been purified to homogeneity using the antibody affinity column chromatography procedure developed by Staehlin *et al.*¹⁸. As shown in Fig. 4B, SDS-polyacrylamide gel electrophoresis of a mixture of purified *E. coli* and yeast LeIF-D produces a single band corresponding to a molecular weight of 20,000. Tryptic maps of purified yeast and bacterial LeIF-D are indistinguishable (R. Wetzel, unpublished results).

Transcription starting and stopping signals

The observation that all six deleted *ADH1* promoters allow appreciable expression of the LeIF-D gene, although their end points vary over a 28-bp region (Fig. 2), indicates the non-essentiality of any particular part of the leader region for synthesis of a translatable mRNA. This finding supports the general model^{19,20} for translation initiation rather than the alternative suggestion that a specific sequence may be required for ribosome binding.

Preliminary data obtained in hybridization experiments between an LeIF-D radiolabelled probe and size-separated *in vivo* transcripts from *ADH1*-LeIF-D fusions suggest that the mRNA for pFRS36 is about 1,500 nucleotides long and that for pFRS35 ~2,100 nucleotides (F.H. and M. Shepard, unpublished results). As the former contains one LeIF-D between the *ADH1* promoter (deletion 906) and *TRP1*, while the latter contains two consecutive LeIF-D coding sequences, these data strongly implicate a site near the 3' end of the *TRP1* gene as the polyadenylation and/or transcription termination signal for *ADH1*-promoted interferon RNA molecules. Further variations in plasmid construction in this system, using 3'-flanking sequences from various yeast genes, should permit direct assessment of the role of termination and polyadenylation in the efficiency of *in vivo* gene expression.

Requirements for heterologous gene expression in yeast

In addition to the need for a promoter sequence from yeast for correct transcription initiation and for the absence of intervening sequences of types not processed in yeast⁶, comparison of the results described here with those from an earlier study (G.A. and A. Barta, unpublished results) in which the complete cDNA sequence coding for rat growth hormone (RGH) prepolypeptide²¹ was fused to a deleted (-30) *ADH1* promoter fragment suggest other requirements for efficient expression of heterologous genes in yeast. In yeast cells transformed with the *ADH1*-RGH fused gene, rat growth hormone mRNA was efficiently and accurately transcribed, starting from the -37 start point, continuing through the RGH coding sequence and terminating discretely in the 2 μ sequences of the vector. Paradoxically, immunological methods detected no synthesis of rat growth hormone polypeptide. Possible significant differences between the productive *ADH1*-LeIF-D fusions and the post-transcriptionally blocked *ADH1*-RGH fusion are the following.

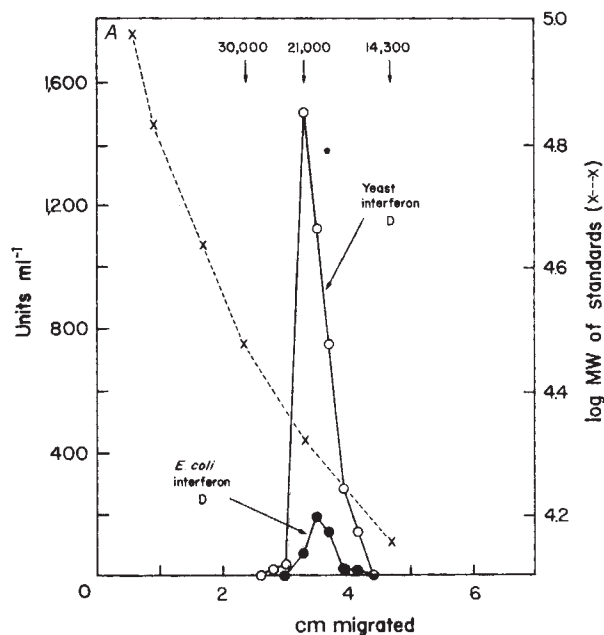


Fig. 4 A, comparison of migration of LeIF-D bioactivity from appropriate yeast and *E. coli* extracts on an SDS polyacrylamide gel. Yeast extracts were prepared as described in Table 1 legend. Bacterial extracts were prepared from *E. coli* 294 (ref. 29) containing the plasmid pLeIF-D trpl by the procedure described in ref. 13. Both extracts were dialysed against 20 mM sodium phosphate, pH 7.0, 0.14 M NaCl, heat treated with SDS and dithiothreitol, and run on a 12.5% SDS-polyacrylamide slab gel using standard methods³⁰. BioRad SDS-polyacrylamide gel electrophoresis standards were used as molecular weight (MW) (14,000–94,000) markers. Standards were stained using Coomassie brilliant blue while the two interferon lanes were sliced into ~0.2-cm strips. The slices were put into assay dilution buffer (see Table 1 legend) for 3 days (to allow diffusion of protein from slices) followed by assay for interferon activity (see Table 1 legend). B, comparison of LeIF-D purified from *E. coli* and yeast, using an antibody column¹⁹ and resuspended in 20 mM sodium phosphate, pH 7.0, 1% glycerol (v/v). After heating with SDS and β -mercaptoethanol, homogeneous proteins were electrophoresed separately and mixed on an SDS-polyacrylamide gel as described above. Interferon was stained using Coomassie brilliant blue. Lane a, 5 μ g LeIF-D purified from yeast; b, mixture containing 2 μ g LeIF-D purified from *E. coli* and 3 μ g LeIF-D purified from yeast; c, 3 μ g LeIF-D purified from *E. coli*.

(1) Signal peptide sequence: Rat growth hormone may indeed have been translated in yeast and been extruded into the rough endoplasmic reticulum²², but it might then have been degraded because of an inability to proceed normally through the yeast secretory pathway. The LeIF-D plasmids used in the present study contain no signal peptide coding sequences, so any interferon made in yeast should have remained in the cytosol.

(2) The sequence context preceding the initiator ATG: For highly expressed yeast genes, nucleotides -1 to -10 in the untranslated leader region of mRNA are typically rich in A with a few Cs and Ts, but no Gs. An A residue is found at position -3 or -4 in all yeast mRNAs to far analysed. The construction used in the expression of interferon from the *ADH1* promoter followed this rule (leader sequence: AATTCATG); that for rat growth hormone gave a leader sequence (GTGCGATG) very unlike those of yeast mRNAs. This unusual leader sequence may not have supported efficient translation initiation by yeast ribosomes.

(3) Codon usage differences: In addition to major differences in arginine codons, which are mainly AGA in LeIF-D and CGPy in RGH, the rat growth hormone leader region is very rich in CUC and CUG leucine codons. These are very rarely used in yeast proteins^{8,23}.

Conclusions and prospects

A mammalian protein, human leukocyte interferon, can be made efficiently in yeast by the fusion of its coding sequence to a yeast promoter and transcription terminator within a yeast

plasmid vector. In such fusions, the yeast signals specifying the 5' and 3' ends of mature mRNA still function predictably after they are grafted onto protein-encoding sequences from another organism. The LeIF-D made in yeast seems to be stable there because it can accumulate to as many as 10^6 molecules per cell. Although the expression levels obtained are high for a preliminary study, improvements may be expected by the use of plasmids with decreased rates of mitotic loss. Alternatively, transformants may be stabilized by chromosomal integration of the hybrid transcription units.

The techniques described here may enable the genetic engineering of yeast to produce many types of protein which normally occur only in other organisms; this may be important for production of pharmaceutical substances and for altered fermentation behaviour of the yeast cells. The yeast system should be particularly advantageous for the synthesis of glycoproteins from higher organisms because the addition of the inner core of carbohydrate occurs by the same mechanism as in animal cells²⁴ and the secretory pathway follows the general pathway used by animal cells²².

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LETTERS TO NATURE

Supermassive binaries in active galactic nuclei

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Recent observational evidence¹⁻⁶ indicating the apparent precession of jets in compact and extended radio sources suggests the presence of a second massive body in the nuclei of the associated active galaxies⁷ (we include quasars as active nuclei). On the basis of their similarities with close binaries in the Galaxy, two of which (SS433 and Her X-1) are known to contain precessing beams and disks, we consider here the possibility that the secondary object is a Roche overflowing supermassive star (SMS) of mass $M_2 \sim 10^6$ - $10^8 M_\odot$ in orbit around a primary massive black hole (MBH) of mass $M_1 \sim 10^7$ - $10^9 M_\odot$.

Jet precession and evolutionary considerations led Begelman, Blandford and Rees⁷ to propose a binary model containing two MBHs in which the observed jet motion is identified with the geodetic precession of the more massive component ($M_1 \sim 10^8 M_\odot$). Although this model can account for the inversion-

symmetric morphology⁵ and apparent precession seen in extended radio jets (such as 3C315, NGC326, 3C196 and 3C305), which indicate precession periods $P_p \sim 10^5$ - 10^8 yr (refs 3, 4, 7), it does not easily explain the $\sim 10^3$ -yr precession periods suggested for the fast-moving compact VLBI features seen in 3C273 and 3C345 (refs 1, 2, 7). The basic problem is that precession periods $\leq 10^3$ yr require orbital separation distances that are short lived due to gravitational radiation, unless implausibly low masses are assumed¹.

Two similar scaled-down relativistic precession models^{8,9} were originally proposed for SS433, but the subsequent discovery¹⁰ of a 13-day orbital period (rather than an expected period of 4-8 min) suggests¹¹ that these relativistic models are probably not applicable to SS433. There are, however, other binary models for SS433 which are simultaneously compatible with a 13-day orbital period and a 164-day beam precession period; two, the slaved disk (SD)¹²⁻¹⁴ and the disk-driven (DD) precession¹⁵ models, will be considered below in the context of a SMS/MBH binary model for active nuclei.

Drawing our analogy from SS433 and Her X-1, we assume that active galactic nuclei which exhibit jet precession are powered by accretion onto a MBH when a companion SMS fills its critical Roche surface. Most of the compact core continuum radiation (optical and flat spectrum radio) is assumed to originate in the accretion disk around M_1 and only a negligible contribution originates with the SMS (compare with SS433 and

cataclysmic variables). Well known luminosity and lifetime arguments suggest that $M_1 \sim 10^7\text{--}10^9 M_\odot$ and $M_2 \sim 10^6\text{--}10^8 M_\odot$. We thus adopt as representative masses $M_1 \sim 10^8 M_\odot$ and $M_2 \sim 10^7 M_\odot$, values also used by Begelman *et al.* We do not speculate here on the evolutionary details for such a binary system, except to note that some aspects of the Begelman *et al.* evolution involving galactic mergers might be relevant and that misalignment between extended jets and the minor axis of elliptical galaxies is sometimes observed. The SMS must form relatively near the critical separation radius where Roche overflow is possible (in which case mass transfer might be initiated by pulsational or rotational instabilities), or have a lifetime sufficiently long that it remains in a quasi-equilibrium while viscous drag or mass loss reduces the orbital separation. After Roche overflow has begun, further mass transfer could be driven by continued orbit decay due to viscous drag or angular momentum loss from the system^{16,17}. Investigations of the stability of SMSs indicate that stars of mass $\sim 10^6\text{--}10^8 M_\odot$ may be stabilized against hydrodynamic collapse for $\sim 10^6\text{--}10^7$ yr by the presence of a modest amount of differential rotation^{18–21}, and by possible contributions from macroscopic turbulence and internal magnetic fields (spinars)^{20,21}. The relatively short lifetime of SMSs may require that their formation be triggered by a merger event.

If the SMS fills its critical Roche surface, then its mean radius is given by^{22,23}

$$\frac{R_2}{r} \approx \frac{0.462}{(1+q)^{1/3}} \quad q \geq 3 \quad (1)$$

where r is the semimajor axis (assumed to be approximately circular) and $q = M_1/M_2$ is the mass ratio. For $q \sim 10$, $R_2/r \sim 0.21$. Thus, in sources with compact cores there is a small but potentially significant probability of eclipsing. Assuming random orientations and disk radii $\leq R_2$, the probability of eclipsing in a given source is ~ 0.07 for $R_2/r \sim 0.21$. Note that this probability is fairly insensitive to q . An estimate of the expected orbital period and possible selection effects are discussed below.

Although the evidence for massive binaries is more direct in the precessing jet sources, in the context of binary models many and perhaps all active nuclei probably contain SMS/MBH binaries. Precessing jets would then be seen only in those sources in which there is misalignment between the orbit normal and the spin axis of M_1 or M_2 . As the beam precession angle θ is not expected to be too large (for example, SS433, $\theta \approx 20^\circ$ and Her X-1, $\theta \sim 5^\circ$), eclipsing should be more probable in compact cores in which the associated jets lie nearly in the plane of the sky.

In the slaved disk model^{12–14,24}, it is assumed that the spin axis $\vec{S}_2$ of the secondary is misaligned with the orbit normal. The observed precession period, P_{SD} , is then associated with the forced precession of the secondary (which has a quadrupole moment by virtue of its rotational distortion) by the primary. The secondary is assumed to fill its critical Roche surface and is losing mass through the inner Lagrange point. As the angular momentum of the transferred matter will depend on the orientation of $\vec{S}_2$, the accretion disk around M_1 will orient itself accordingly. If the transferred material is processed through the disk in a time that is short compared with the precession period of $\vec{S}_2$, then the disk orientation will follow $\vec{S}_2$. The disk is thus coupled to or ‘slaved’ to the precessing axis of the secondary star²⁴. As in the case of SS433, we assume that the beams are ejected normal to the surfaces of the precessing disk^{12–14}. Note that Linfield¹ has independently suggested an accretion disk as the source of the $\sim 10^3$ -yr precession period in the compact jets in 3C273 and 3C345.

The problem of long-lived forced precession in a fluid star was noted by Roberts²⁴ who indicated that different shells may precess at different rates, leading to complex internal motions. Superficially, such differential precession might seem to lead to alignment on a relatively short time scale. However, forced

precession may be maintained if the precessing modes involve radial motion and circulation in the star²⁵. Other authors have concluded that long-term forced precession is possible²⁶ and that the alignment time scale can be comparable with or longer than the synchronization time scale²⁷.

The forced precession rate of the SMS is given by^{23,14}

$$\frac{\Omega_{SD}}{\omega_0} \approx \left(\frac{k'}{k}\right) \left(\frac{\omega_2}{\omega_0}\right) \left(\frac{R_2}{r}\right)^3 \left(\frac{M_1}{M_2}\right) (1-e^2)^{-3/2} \quad (2)$$

where k' is the apsidal motion constant, k the gyration parameter, e the eccentricity, r the semimajor axis, ω_0 the orbital angular velocity and ω_2 the angular velocity of the secondary. Assuming an approximately circular orbit and synchronous rotation, we may substitute R_2/r from equation (1) into equation (2), yielding $\Omega_{SD}/\omega_0 \sim 0.1(k'/k)$, which is independent of M_1 , M_2 and q provided $q \geq 3$. For a SMS model with polytropic index $n = 3$ (refs 18, 19), we may use the table given by Motz²⁸ to obtain $k'/k \approx 0.19$, which is consistent with the estimate¹⁴ of $k'/k \leq 1/3$ for $n \geq 2.5$. Inserting this into equation (2) and setting $\Omega_{SD} \sim 2\pi$ per 10^3 yr gives an orbital period of $P_0 \sim 20$ yr. For $M_1 = 10^8 M_\odot$, $q \geq 3$ and $e \approx 0$, the corresponding binary separation distance is $r \sim 5 \times 10^{16}$ cm. The relativistic parameter, $2GM_2/c^2 R_2$, is $\sim 3 \times 10^{-4}$. Thus the SMS could easily be supported by an expected modest amount of differential rotation and magnetic field.

An orbital period of ~ 20 yr is potentially observable in sources such as 3C273 and 3C345 which exhibit $\sim 10^3$ -yr precession periods. Unfortunately, selection effects may enhance the probability of observing non-eclipsing compact sources. Relativistic jets that are oriented nearly towards the observer will be Doppler enhanced and more easily detected^{1,7}, such as the asymmetric compact superluminal jets in 3C273 (ref. 29). But compact cores with transverse jets are more likely to exhibit eclipsing because the beam precession angle is expected to be relatively small. On the other hand, the basic SMS/MBH binary model may be applicable to sources in which there is no misalignment. Consequently, our eclipsing probability estimate of ~ 0.07 may be relevant to most active nuclei.

If the slaved disk model is applicable to the extended jets with precession periods $\sim 10^5\text{--}10^8$ yr (refs 1, 3, 4, 7), then the implied orbital periods are $\sim 2 \times 10^3\text{--}2 \times 10^6$ yr, which are obviously not directly observable. However, if these extended jets are subrelativistic, then their curvature may not be due to precession¹ and the SD model is not applicable. In this case, active nuclei associated with the extended curved jets, as well as the extended linear jets, may still potentially exhibit eclipsing (but of less certain period) if the basic SMS/MBH binary model is relevant. Consequently, sources with extended jets lying near the plane of the sky might constitute a preferred sample in which relativistic beaming effects would be much less important.

Comparing the ratio of orbital period to precession period in the SD and geodetic models (Begelman *et al.*) gives $(P_{SD}/P_{geo}) \sim 2.5 \times 10^{-3}$ for $M_2 \sim 10^7 M_\odot$ and $r \sim 5 \times 10^{16}$ cm. The gravitational radiation time scale (Begelman *et al.*) for the SD model parameters ($M_1 \sim 10^8 M_\odot$, $M_2 \sim 10^7 M_\odot$ and $r \sim 5 \times 10^{16}$ cm) is $t_{GR} \sim 2 \times 10^9$ yr. Thus in the SD model, the system is not seriously unstable to gravitational radiation. Begelman *et al.* estimate a precession period of ~ 500 yr for the fast-moving jets in 3C273 and 3C345. However, their suggested parameters of $M_1 \sim M_2 \sim 10^8 M_\odot$, $r \sim 1.7 \times 10^{16}$ cm and $t_{GR} \sim 10^6$ yr are inconsistent with a precession period of 500 yr (compare with their equation (8) which gives a precession period of $\sim 2.3 \times 10^3$ yr). Although these parameters are intended only as order-of-magnitude estimates and might be adjusted to ensure consistency, this illustrates the difficulty in obtaining the required precession periods with geodetic precession, as noted elsewhere¹. (Lens Thirring and Kerr-quadrupole-moment precession of M_1 by M_2 is even less effective.) The geodetic model can, however, easily account for the extended jet precession periods.

The disk-driven precession model (DD) has also been

proposed¹⁵ to account for SS443 and might be relevant to precession in extragalactic jets. In this model, a stationary massive outer disk around the compact object causes the compact object's spin axis $\hat{S}_1$ (plus the inner portion of the disk) to precess due to the Lens Thirring effect. From angular momentum considerations, a critical radius, r_p , is identified such that disk material at radii $\geq r_p$ is not precessed by the compact object plus inner disk while material interior to r_p is aligned with the precessing equatorial plane of the compact object. It is assumed that accreting matter is collimated and ejected out along $\hat{S}_1$, which is also the direction normal to the inner disk^{8,30,31}.

Applied to SS433, the DD model requires a radial inflow rate of $v_r/v_\theta \sim 10^{-10}$ – 10^{-9} , corresponding to a disk viscosity parameter of $\alpha \sim 10^{-9}$ – 10^{-7} at r_p ¹⁵. This very small inflow rate is the opposite extreme of the more conventional rate required for the SD model¹⁴ and the two models are therefore mutually exclusive. However, the required inflow rate is less extreme for disks around MBHs. The formulae given by Sarazin *et al.*¹⁵ refer to a $1 M_\odot$ compact object. Inserting the M_1 dependence into their equation (5) and using the appropriate angular momentum for an extreme Kerr black hole leads to an inflow rate of $v_r/v_\theta \sim 10^{-5}$ at r_p , assuming $M_1 \sim 10^8 M_\odot$, $P_{LT} \sim 10^3$ yr and $M/M(\text{Eddington}) \sim 500$. The latter assumption is appropriate for SS433 but may be an overestimate here. If $P_{LT} \sim 10^7$ yr, $v_r/v_\theta \sim 10^{-2}$ for the same parameters.

The required disk mass could most easily be obtained by Roche overflow from a companion SMS by analogy with SS433, Her X-1 and numerous other galactic binaries with compact objects and accretion disks. In this case, eclipsing may occur as previously discussed, but it is not possible to obtain a simple relationship between the orbit and precession periods. If the necessary supercritical accretion and inflow rates are realizable, there might be a weak statistical relationship between the precession period and luminosity (as reflected by $\dot{M}$) because $\Omega_{LT} \propto \dot{M}^{3/2}$.

Clearly all three models (geodetic precession, slaved disk and disk-driven) can accommodate precession in extended sources ($P_p \sim 10^5$ – 10^8 yr), but only the slaved disk model can easily explain the short period ($\sim 10^3$ yr) precession indicated by VLBI observations of fast-moving jets in 3C273 and 3C345 (refs 1, 2). All three models have been proposed to explain the 164-day beam precession period in SS433, a binary system now believed to consist of a relatively normal Roche overflowing star and a compact object^{32,33}. The compact binary system Her X-1/HZ Her is known to contain a large precessing disk, and therefore of the three models considered only the slaved disk model is applicable to this system. There are, of course, numerous other examples of (non-misaligned) galactic binary systems which are radiating near their Eddington limit due to Roche overflow from a normal star onto a compact object. Thus active galactic nuclei, especially those suggesting jet precession, may be scaled-up versions of these better understood galactic binaries. Recently, Neill *et al.*³⁴ have concluded that their VLBI observations of SS433 favour the kinematical precessing jet model of Linfield¹ for the superluminal jets seen in 3C273 and 3C345 over the density gradient model of Readhead *et al.*³⁵.

In the context of the slaved disk model, orbital periods of ~ 20 yr are expected in compact sources with $\sim 10^3$ -yr precession periods. If the jets and compact core continuum originate in the accretion disk, then the probability of eclipsing should be greatest in compact sources whose associated fast-moving jets lie near the plane of the sky, although selection effects due to relativistic beaming may discriminate against this geometry. The basic SMS/MBH binary model may also be relevant to active nuclei which do not exhibit precession because of a lack of misalignment, in which case $\sim 7\%$ of all active compact cores could potentially be eclipsed. The observational probability might be improved by choosing a sample which includes compact central cores that are associated with the more common extended jets (as well as any compact jets) which lie close to the plane of the sky.

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Laboratory produced visible spectral emission features correlate with those of the Red Rectangle

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Warren-Smith *et al.*¹ and Schmidt *et al.*² have obtained peculiar optical spectra of the Red Rectangle, which is a nebula centred on the star HD44179 that possesses the geometry of a hollow biconical shape with the star at the apex. This is also the source of IR emission features at 3.3, 6.2 and 7.7 μm (refs 3, 4). Warren-Smith *et al.*¹ report the presence of R-type band-like features, characterized by a profile of sharp rise from shorter wavelengths and a gradual degradation to the red, occurring at $\lambda 5,779$, 5,855, 5,880 and 6,615, with the first two and the last being the most intense. They attribute the origin of these emissions to unsaturated carbon molecules or carbynes. I report here that matrix isolation experiments directed towards understanding the origin of the diffuse interstellar bands also show spectral emission features having characteristics similar to those found in the visible spectrum of the Red Rectangle nebula. These experiments suggest that the observed Red Rectangle emissions have their origins in fluorescence phenomena of species that are matrix-isolated in grain mantles.

From a 0.5% methane in argon plasma, I have produced a species having a spectral absorption pattern that correlates with the principal diffuse interstellar bands⁵. The products of the discharge are trapped in an argon matrix at $T < 20\text{K}$ and spectroscopic measurements made by transmitting through the sample light from an 80-W tungsten lamp (DGB; estimated filament temperature 3,150K) used with a Kodak heat rejection filter having decreasing transmission between $\lambda 3,750$ and $\lambda 3,250$.

Besides the absorption features described in ref. 5, the spectra exhibit four fluorescence features excited by the tungsten lamp. One of these features is probably R-line fluorescence of chromium ions as impurities in the sapphire substrate

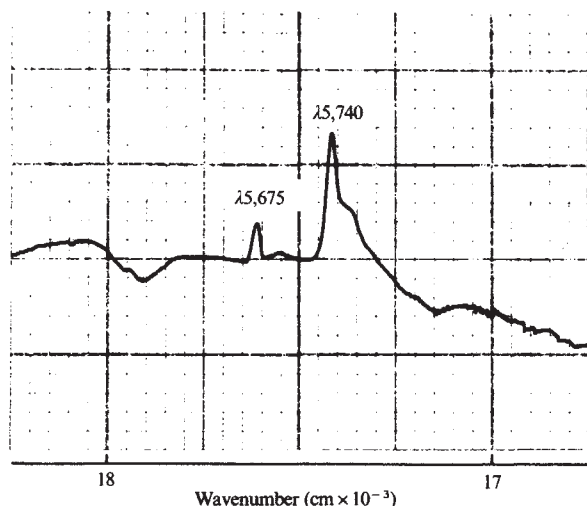


Fig. 1 Single-beam spectrophotometric tracing of $\lambda 5,740$ and $5,675$ emission features under illumination from an 80 W tungsten lamp. 11-h deposition at 11K and spectrum obtained at 12K. Thickness of the sample was estimated to be 2 mm.

(D. O'Shea, personal communication). The other features (Fig. 1) at $\lambda 5,740$ ($17,420 \text{ cm}^{-1}$) and $5,675$ ($17,620 \text{ cm}^{-1}$) are related to a reaction product or products of the discharge. The stronger feature at $\lambda 5,740$ ($17,420 \text{ cm}^{-1}$) shows the same profile as the R-type emission features observed from the Red Rectangle. In some experiments, the profile of the weaker feature ($\lambda 5,675$) suggests similar behaviour. A third weak emission feature was observed in some experiments at $\lambda 6,382$ ($15,670 \text{ cm}^{-1}$)—this was seen after 8–10-h depositions and correlates with the Red Rectangle M-type feature at $\lambda 6,378$. The strengths of the emission features seem to be correlated with absorption features attributed to C_2^- . They are strongest when C_2^- absorption is strongest, and disappear along with C_2^- under UV irradiation from a mercury vapour lamp. However, the redward portion of the profile diminishes more slowly than the peak, and continues to exist after C_2^- absorption has been bleached away. The two emissions at $\lambda 5,740$ and $5,675$ may be due to a single species existing at two different kinds of site, resulting in the splitting of what would have been one feature: such behaviour is not uncommon in matrix isolation.

The wavelengths, profiles and means of production of these three laboratory features suggest that they are correlated with the emission features of the Red Rectangle. The same or similar species may be matrix-isolated in the mantles of grains in the process of exposure to illumination by HD44179. Diminishment of emission under UV irradiation as demonstrated in the experiments requires that the same or similar species, if present in the Red Rectangle, be shielded at least temporarily from the photolysing portion of the UV radiation emitted by the early-type central star. This might be accomplished by extinction due to inner shells of circumstellar grains. The differences in wavelengths of less than 2% may be explained by the species being in a matrix having different characteristics than those of argon. For example, the strongest of the C_2^- absorption features undergoes a $\Delta\lambda = 95$ (or 1.8% difference in wavelength) between isolation in argon and neon matrices⁶. Note that methane ice in a mantle matrix has been suggested as being responsible for the $3.3\text{-}\mu\text{m}$ emission feature of the Red Rectangle and NGC7027 (ref. 7).

The production of emission features in the laboratory as described here, and the observed celestial features, suggest the possibility of using the species responsible as a probe of the nature of grain mantles. Experiments involving matrices having the characteristics expected of the mantles of interstellar grains are of interest from the standpoint of adjusting the emission features with varied matrix compositions, to shift them into exact coincidence with the observed phenomena.

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Organic synthesis in the atmosphere of Titan

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Titan, the largest satellite of Saturn, is one of the smallest known bodies with an atmosphere. Since the detection of an atmosphere on Titan by Kuiper, various models have been proposed for the structure of this atmosphere¹. These models were based on information from ground-based observations which, although advanced, failed to yield a sufficiently accurate and quantitative picture of the atmosphere. The IR, UV and radio science investigations on Voyager 1 have now provided much needed information on the composition, structure and dynamics of the atmosphere of Titan². The IR and radio science data indicated a mean molecular weight of 28 for the atmosphere of Titan and it was thus concluded³ that the atmosphere consists predominantly of N_2 . The minor components of the atmosphere as identified by the spectra from the IR Radiometer and Interferometer Spectrometer (IRIS) spectra include methane, acetylene, hydrogen cyanide, ethane, ethylene and possibly propane and methylacetylene³. The other IR bands observed in the spectra have not been firmly identified, although several other hydrocarbons and nitrogen-containing compounds are suspected. The minimum atmospheric temperature of $\sim 70 \text{ K}$ was deduced to prevail near the 200 mbar level, which can act as a cold trap for methane and thus regulate and maintain a constant abundance of stratospheric CH_4 , which has been estimated³ to be about 1% of N_2 . The IRIS data also indicate that the stratospheric temperature near 1 mbar level is about 20 K colder in the north than at the equatorial and south latitudes but that it shows practically no diurnal and longitudinal thermal variation.

Speculation on the processes which could contribute to the organic chemistry of Titan depend on the selection of the atmospheric model. The ground-based observation of C_2H_6 , C_2H_4 and C_2H_2 , along with the coloration of the planet, led to several competing models for the atmosphere. At one-extreme, the temperature inversion model^{4,5} envisages a thin atmosphere (surface pressure and temperature of ~ 20 mbar and 80 K, respectively), which is heated to 160 K by dust (aerosol) which absorbs the UV and visible radiation. The greenhouse model^{6,7}, on the other hand, assumes a thick atmosphere (upper limits of surface pressure 21 bar and temperature 200 K) consisting mainly of N_2 with CH_4 and H_2 as minor constituents. A continuous opacity is provided by dust particles dissolved in liquid methane droplets in this model.

A pre-Voyager report on the organic synthesis on Titan by Chang *et al.*⁸ is based on a composite model. The main features of this model are: (1) the absorption of solar radiations in the

stratosphere by a dust layer; (2) the temperature in the stratosphere is ~ 17 K, which drops to ~ 115 K at the top of a thick, dust-covered cloud layer consisting of colourless hydrocarbons mixed with reddish material of unknown composition and possibly solid ammonia; (3) the main constituents of the atmosphere are N_2 and CH_4 ; (4) a clear atmosphere extends from the bottom of the clouds to the surface with temperature of ~ 135 K and pressure of 1 bar; (5) the surface consists (mainly) of CH_4 clathrates, NH_3-H_2O ice or liquid NH_3 , and therefore the abundance of NH_3 in the atmosphere is regulated by its saturation vapour pressure.

The situations assumed by these models are substantially different from the actual one which can be formulated by the Voyager data, especially with regard to temperature, pressure, composition and dust opacity. To gain some insight into the organic chemistry on Titan, in the light of the recent data provided by Voyager, we carried out several simulation experiments. Our main objective was to assess the possible contributions to the organic chemistry from different possible energy sources which may be available on the satellite.

In our experiments, a gas mixture of N_2 and CH_4 ($\sim 100:1$, occasionally up to $100:4$), simulated Titan's atmospheric composition as revealed by the Voyager data. The energy sources used were UV radiation, electric discharges, an electron beam, γ -ray radiation and a proton beam.

There is no question that UV and visible radiation from the Sun is available. Although Voyager 1 did not observe directly any evidence of lightning in Titan's atmosphere, its occurrence remains a possibility. The seasonally dependent cyclostrophic zonal flow in the stratosphere of about 100 m s^{-1} might be of great significance in this regard³. High-energy particles from cosmic rays, medium-energy particles from solar winds and low-energy particles from Saturn are also a very likely source of energy on Titan. The low energy bombardment will have its primary role in the stratosphere while the cosmic rays can contribute to the organic synthesis in the deeper atmosphere. Titan seems to move in and out of Saturn's magnetosphere. The magnetometric measurements by Voyager showed the absence of an internal magnetic field on Titan⁹, and therefore the solar wind as well as high-energy particles trapped in Saturn's magnetosphere can reach Titan nearly unimpeded. We have examined the roles of each of these energy sources in the organic synthesis on Titan by laboratory simulation experiments.

(1) Ultraviolet irradiation: In one experiment, a mixture of CH_4 and N_2 ($1:100$, a total pressure 505 mbar) was photolysed with a high pressure (15 atm, 17 kW) argon lamp for 4 h. In another experiment, the flask containing 500 mbar of N_2 was equipped with a CH_4 reservoir which was dipped in a liquid nitrogen Dewar, thus regulating and replenishing the CH_4 supply. After the UV irradiation, the bulk of N_2 remaining was pumped out and the gas products were subjected to gas chromatographic (LGC) and mass spectroscopic (MS) analysis. Both experiments yielded virtually identical results. About $30\text{ }\mu\text{mol}$ of C_2H_6 , $2\text{ }\mu\text{mol}$ of C_3H_8 and $1\text{ }\mu\text{mol}$ of C_2H_4 per mol of CH_4 were produced. Acetylene and hydrogen cyanide, if present, occurred at levels $<10^{-2}\text{ }\mu\text{mol}$. Note that this result is associated with the wavelength between 100 and 200 nm provided by the argon source.

(2) Electric discharge: A mixture of 10 mbar of CH_4 and 740 mbar of N_2 was subjected to an electric discharge ($\sim 5,000$ V tungsten electrodes, ~ 1 cm gap). Within 15 min, C_2H_2 and HCN could be observed by IR spectroscopy. A pale brown film began to be deposited on the walls of the vessel and also on the windows of the IR cell attached to the vessel, on further continuance of electrical discharge. The gaseous products were sampled after 15 min and 1, 2, 6 and 12 h and were then analysed by GC and GC/MS. All the compounds identified in Titan's atmosphere were detected in these samples. However, compared with the situation on Titan the production of C_2H_2 was much higher than that of C_2H_6 . Other products such as diacetylene, cyanoacetylene and ethylene were clearly observed by GC and positively identified by GC/MS from the

Table 1 Relative abundance of products in simulated Titan's atmosphere

UV	Electric discharge	γ -Ray radiation	Proton beam	Electron beam
C_2H_6 (primary product)	HCN, C_2H_2 (primary product)	HCN, C_2H_2	HCN	HCN
$\gg C_2H_4$	$>C_2H_4$	$>C_2H_4$	$>C_2H_2$	$>C_2H_2$
$\gg C_3H_8$	$>C_3H_6$	$>C_3H_2$	$>C_3H_4$	$\gg C_3H_4$
	$>C_4H_2$	$>C_3HN$	$>C_4H_2$	$>C_4H_2$
	$>C_3HN$	$\gg CH_3CN$	$>C_3HN$	$>C_3HN$
	$>C_3H_6$		$>C_3H_4$	$\gg C_3H_6$
	$\gg C_3H_8$		$>CH_3CN$	$\gg CH_3CN$
	$\gg CH_3CN$		$NH_3?$	

$>$ Approximately an order of magnitude less; $\gg$ one to two orders of magnitude less.

fragmentation patterns. If lightning does occur on Titan, all the observed components of Titan's atmosphere can be accounted for by this simulation experiment. The brownish haze which deposits on the IR cell windows during the reaction is practically transparent in the $500\text{--}600\text{ cm}^{-1}$ region, where the hydrocarbon aerosols are likely to absorb. Such a haze, if present on Titan, will impart to its observed colours without much interference in the $300\text{--}1,000\text{ cm}^{-1}$ region.

(3) γ -ray irradiation: A mixture of 10 mbar CH_4 and 700 mbar N_2 was exposed to radiations at the rate of $\sim 20\text{ krad h}^{-1}$ for 40 h using a ^{60}Co source. C_2H_2 and HCN were the prime products followed by C_2H_4 , C_4H_2 , C_3HN and a few unidentified compounds.

(4) Electron bombardment: A 2-l flask equipped with a CH_4 reservoir maintained at 77 K was filled with 700 mbar of N_2 . The mixture was bombarded with a diffuse 10-MeV electron beam (20 nA, dosage $\sim 20\text{ krad s}^{-1}$) for 50 min. C_2H_2 , C_2H_4 , C_3H_6 , C_4H_2 , CH_3CN , HCN and C_3HN were positively identified by GC. A few more compounds were formed in very low concentrations.

(5) Proton bombardment: A mixture (total pressure of 2 mbar) of N_2 and CH_4 ($100:4$) was contained in a cell. A proton beam ($3 \times 10^{-8}\text{ A}$, 1.5 MeV) was allowed to pass through a 3×10^{-7} inch-thick pure nickel foil window. The reaction was carried out for 3 h. GC and GC/MS data of the resulting sample gas mixture indicated the presence of C_3H_4 , C_2H_2 , C_2H_4 , HCN, C_4H_2 and C_3HN ; the last two compounds were present in smaller quantities than the others. An attempt to detect NH_3 by mass spectrometry was inconclusive due to interference with CH_4 .

The experimental results obtained are summarized in Table 1. They indicate that (1) UV light produced by the argon lamp produces saturated hydrocarbons such as C_2H_6 and C_3H_8 but no appreciable amount of unsaturated hydrocarbons such as C_2H_4 and C_2H_2 and nitrogen-containing compounds, especially HCN; (2) electric discharge and radiation by γ -ray, β - and proton particles produce HCN and more unsaturated hydrocarbons, especially C_2H_2 , than saturated hydrocarbons.

C_2H_2 is believed to be formed from C_2H_6 or C_2H_4 in CH_4 photolysis. The main source of acetylene in C_2H_6 photolysis is the species C_2H_4 , which dissociates into $C_2H_2 + H_2$. This process of production of C_2H_2 is retarded in the presence of nitrogen N_2 (ref. 10). In other words, C_2H_2 can be produced by photolysis of CH_4 by a route $CH_4 \rightarrow C_2H_6 \rightarrow C_2H_4^* \rightarrow C_2H_2$. Because the production rate of C_2H_6 , the first intermediate, is not very large and nitrogen is present in a large excess in Titan's atmosphere, it is very unlikely that C_2H_2 is produced in a significant amount by UV irradiation alone. Moreover, to obtain an appreciable steady-state level of C_2H_2 , any process of C_2H_2 production must be more efficient than the photodissociation of C_2H_2 which has a high absorption coefficient below 160 nm (refs 11, 12).

Chang *et al.*⁸ also failed to observe any HCN on irradiation of a mixture of CH_4 and N_2 with a 123.6-nm lamp. However, Laufer and Bass¹³ reported HCN production at ~ 160 nm from 3CH_2 radical and nitrogen. Lyman α photons in Titan's atmosphere can produce 1CH_2 radical which may thermalize by an intersystem crossing to 3CH_2 but only at a very small rate of

$9 \times 10^{-13} \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ (ref. 14). At higher N_2 pressures, the $^3\text{CH}_2$ concentration is increased¹⁵, indicating that N_2 may act as a rather inert catalyst. However, $^3\text{CH}_2$ reacts with itself (producing C_2H_3) at a two orders-of-magnitude higher rate ($5.3 \times 10^{-11} \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) than its production from CH_2 . The absorption cross-section of HCN increases many fold below 160 nm, implying that HCN is not stable to irradiation below 160 m, and therefore that a significantly steady-state production of HCN by Lyman α -photons from CH_4 and N_2 in Titan's atmosphere at a pressure level of 10 mbar seems improbable. Another possible path to the formation of HCN and other nitrile compounds from N_2 and CH_4 mixture should involve an initial formation of N atoms (from N_2). This is very unlikely with the kind of solar radiation energy Titan seems to be receiving. Scattergood¹⁶ used a 1:1 mixture of CH_4 and N_2 in a proton bombardment experiment, which produced an appreciable amount of NH_3 , and other products similar to those reported by us. In the present experiment, in which hydrogen and CH_4 are rather depleted, it is unlikely that any appreciable NH_3 is produced.

It is possible that the C_2H_2 and HCN found in Titan's atmosphere are produced, not by solar UV radiation, but rather by either lightning or particle or β -radiation, or all of them. The production of NH_3 and other amines is yet to be explored carefully. These could lead to formation of HCN and other nitrogen-containing compounds. The colouration of the planet may be a result of the polymeric products obtained from photolysis of HCN (ref. 17). This seems more likely than C_2H_2 polymers, which have to be highly polymerized to show a visible colour.

In all our laboratory experiments, the production of C_2H_6 was very low, whereas the band around 830 cm^{-1} in the IRIS spectra, which has been assigned³ to C_2H_6 , is substantial. Even if the C_2H_6 produced by solar UV radiation as shown by the UV experiment is taken into consideration the peak at 830 cm^{-1} seems to be unduly strong. The IR spectrum of solid HCN we obtained exhibits a strong broad signal centred around 825 cm^{-1} , along with weak absorption bands at 950 and 740 cm^{-1} . Thus, the band observed around 830 cm^{-1} could be a composite of C_2H_6 and solid HCN suspended in the atmosphere, and the content of C_2H_6 in Titan's atmosphere may not be as high as initially assumed³.

The observation of HCN in the atmosphere of Titan and the corroboration by laboratory simulation experiments are significant in our understanding of chemical evolution. A precursor of biologically important compounds, HCN has been detected in interstellar medium¹⁸ and its derivatives, such as cyano-polyacetylenes and polynitriles, have been suggested to be present in meteorites. The presence of this key compound on Titan implies that the chemical processes postulated as involved in the formation of bases and amino acids on the primitive Earth may be commonplace in the solar system.

A revised upper limit (R. Hanel, personal communication) of the methane content in Titan's stratosphere is 2.6% (of N_2), rather than 1% as reported earlier³. As our experimental condition was such that CH_4 content ranged over 1–4%, the results of the simulation experiments should still be valid, at least for the overall qualitative picture.

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Why olivine transforms to spinel at high pressure

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Because of its importance in understanding the static and dynamic properties of the Earth's mantle, the olivine to spinel structure transformation is one of the most frequently studied crystal structure changes. The volume change is considerable (~7–10%, depending on the compound involved) but the reason for this is unclear. In most high-pressure transformations, the volume contraction is accompanied by an increase in the primary coordination number of the atoms. However, there is no such increase in the case of the transformation from the olivine to the spinel structure. Both are A_2BX_4 compounds (for example, Mg_2SiO_4 olivine and Al_2MgO_4 spinel) with cation coordinations AX_6 (octahedral) and BX_4 (tetrahedral) and anion coordinations XA_3B (tetrahedral). Indeed the conventional description of both structures starts with approximately 'close-packed' ('eutactic') anion arrays (hexagonal for olivine and cubic for spinel) with cations in one half of the octahedral interstices and one eighth of the tetrahedral interstices, suggesting that the volumes of each structure should be very similar for a given compound. Here we show that a less conventional, but more appropriate description of the structures resolves the volume problem and also sheds light on the crystal chemistry of these and related structures.

As there is no change in nearest-neighbour configurations we must examine next-nearest neighbour arrangements. For the anions, both arrays are eutactic and hence of equal packing efficiency. However, when we compare the cation arrays in the two structures there is a considerable, highly significant change.

Figure 1 shows the structure of a typical olivine² drawn in an unconventional way. The right-hand part shows the SiO_4 tetrahedra and Mg atoms and the left-hand part, only the cation array. We emphasize the latter, which consists of Si-centred Mg_6 trigonal prisms connected in such a way that each SiMg_6 prism has three Mg caps, that is, the coordination of Si by Mg is SiMg_9 , although not all nine magnesium atoms are equidistant from the Si. In $\alpha\text{-Fe}_2\text{SiO}_4$ (the olivine form) the analogous distances are $d(\text{Si} \dots \text{Fe}) = 2.785(2\times), 2.878, 3.287, 3.299(2\times), 3.318(2\times)$ and $3.268 \text{ \AA}$ (average = $3.147 \text{ \AA}$). This array is that of Ni_2In (Strukturbericht type B8_b)—a common alloy structure type. The projection of Fig. 1 corresponds to that of Ni_2In on $(11\bar{2}0)^3$.

Figure 2 shows the spinel structure of $\gamma\text{-Fe}_2\text{SiO}_4$ (ref. 4). The cation array is emphasized and again shown to be a common alloy structure type, now MgCu_2 (C15). The Si atoms are in the centres of Fe_{12} truncated tetrahedra (Friauf polyhedra) with $d(\text{Si} \dots \text{Fe}) = 3.414 \text{ \AA}(12\times)$.

Thus the important change in the transformation of olivine to spinel is the change in the A_2B (cation) arrangement from Ni_2In

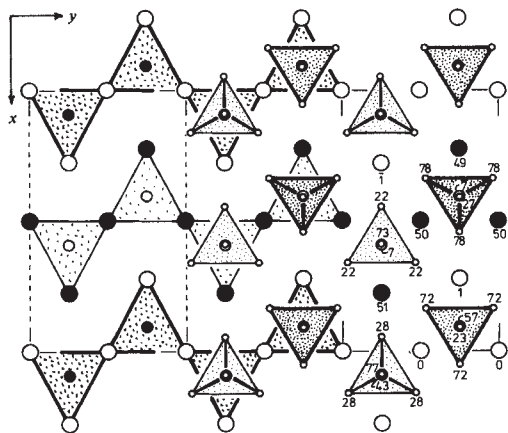


Fig. 1 The structure of olivine (Yosemite 103-481)⁴ projected on (001) (setting Pnma). The right-hand part of the drawing shows SiO₄ tetrahedra and Mg atoms (large circles). Numbers are heights (100z). The SiMg₆ trigonal prisms are shown on the left. These are connected into columns parallel to the projection axis by sharing triangular faces, and the columns are joined into corrugated sheets by sharing prism edges. Alternate sheets are at heights differing by $z = 1/2$.

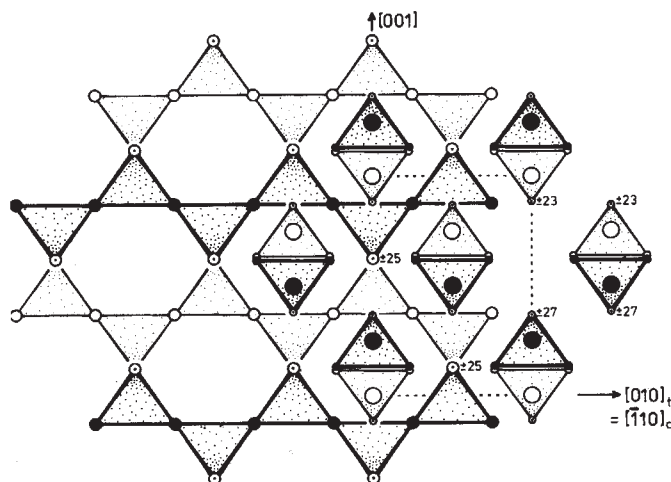


Fig. 2 The spinel structure of $\gamma\text{-Fe}_2\text{SiO}_4$ projected on $(100)_t = (110)_c$. (Subscript c refers to the conventional face-centred-cubic unit cell, t to the corresponding body-centred-tetragonal cell related to c by the matrix $\begin{pmatrix} 1/2 & 0 & 0 \\ 0 & 1/2 & 0 \\ 0 & 0 & 1 \end{pmatrix}$). Large circles are Si, medium circles Fe and small circles O; open at $x_t = 0$, filled at $x_t = 1/2$, dotted at $x_t = \pm 1/4$ (numbers are heights, 100 x_t). Left, the C9-like array of empty, corner-connected Fe₄ tetrahedra; right, the SiO₄ tetrahedra.

Self-exciting dynamos and geomagnetic polarity changes

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Cosmical magnetic fields are produced and maintained against the effects of ohmic decay by inductive interactions with fluid motions. But neither a steady nor a non-steady magnetic field can be supported by this so-called 'self-exciting homogeneous dynamo' process if the field possesses an axis of symmetry. This suggests that palaeomagnetic and archaeomagnetic data might be expected to show evidence that departures from axial symmetry are systematically less during the decay phase of a geomagnetic polarity 'reversal' or 'excursion' than during the growth or recovery phase. The recent proof that neither fluid compressibility nor thermoelectric effects of the Nernst-Ettinghausen type can prevent the ohmic decay of axisymmetric magnetic fields is also discussed here.

The magnetic fields of the Earth and Sun, and of other magnetic planets (such as Jupiter and Saturn) and stars, are thought to be due to electric currents flowing within their interiors. It is accepted that these currents are maintained against the effects of ohmic dissipation largely by electromotive forces (e.m.f.s) due to motional induction, as Larmor¹ first pointed out. The fluid motions involved are produced in most (if not all) cases by the action of gravity on density variations. There have been other general proposals as to the nature of these e.m.f.s but, unlike Larmor's 'self-exciting homogeneous dynamo' proposal, they invariably fail, usually by several powers of 10, to give the right magnitude. Thus, the dynamo mechanism is considered to hold the greatest promise of explaining cosmical magnetic fields, and this is why over the past two or three decades much mathematical work has been done on investigating how such dynamos might work in detail^{2,3}.

The mathematical analysis of theoretical dynamo models is complicated by the finding that suitable departures from axial symmetry seem to be required for dynamo action^{2,3}. Indeed, Cowling⁴ showed that no steady magnetic field with an axis of symmetry can be maintained by fluid motions, and it was later shown that no non-steady axisymmetric magnetic field can be so maintained when the electrically conducting fluid is incompressible^{5,6}. But until recently the behaviour of axisymmetric magnetic fields had not been established in the most general case, when the fluid can be compressible, the field non-steady and the scalar coefficients of electrical conductivity and magnetic permeability dependent on position and time. This led Todoeschuck and Rochester⁷ to speculate that it might be possible for a non-steady axisymmetric magnetic field to be maintained by dynamo action provided the fluid were sufficiently compressible. But it can be shown, giving careful consideration to the definition of dynamo action⁸, that in the most general conditions fluid motions cannot prevent the ohmic decay of an axisymmetric magnetic field⁹⁻¹¹. Any search for axisymmetric dynamos is bound to be unprofitable.

Another speculation which has motivated efforts to generalize Cowling's original 'anti-dynamo' theorem is Hibberd's¹². He proposed that non-steady axisymmetric magnetic fields can be maintained by thermoelectric effects of the Nernst-Ettinghausen type, where the conductive flow of heat down a temperature gradient ∇T in the presence of a magnetic field $\mathbf{B}$ generates an e.m.f. equal to $\mathbf{n} \times \mathbf{B}$ where $\mathbf{n} = -\alpha \nabla T$ if α is the Nernst-Ettinghausen coefficient. Such effects can be included in the analysis of the behaviour of axisymmetric fields and shown to have no significant influence⁸⁻¹⁰. Thus despite speculations to the contrary^{7,12}, neither compressibility nor

to MgCu_2 with an increase in coordination number (BA_6 to BA_{12}) exactly as expected for a high pressure transformation. Equally important is the fact that $d(\text{Si} \dots \text{Fe})$ increases in the high-pressure phase, strongly suggesting that the driving force for the transformation is a lowering of the Si...Fe non-bonded^{5,6} repulsion energy. (It is not generally recognized, but it is, nevertheless, the case, that when coordination numbers increase in a phase transition, so also do interatomic distances.) This change is consistent with the hypothesis, described in detail elsewhere^{5,6}, that cation...cation non-bonded interactions are far more important than those between the oxygens in oxides.

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Nernst–Ettinghausen effects can prevent the ohmic decay of an axisymmetric field.

Application of the laws of Gauss, Faraday and Ampère, together with Ohm's law, to a moving medium in which Nernst–Ettinghausen e.m.f.s are present leads to the following differential equation for the magnetic field $\mathbf{B}$ (see ref. 8):

$$\partial \mathbf{B} / \partial t = -\nabla \times (\sigma^{-1} \nabla \times (\mu^{-1} \mathbf{B}) + \nabla \times ((\mathbf{u} + \mathbf{n}) \times \mathbf{B})) \quad (1)$$

Here t denotes time, σ electrical conductivity, μ magnetic permeability and $\mathbf{u}$ the Eulerian flow velocity. When $\mathbf{B}$ possesses an axis of symmetry its meridional components (B_r, B_z) (in cylindrical coordinates (r, ϕ, z) about the axis of symmetry $r = 0$) and azimuthal component B_ϕ (in general non-zero) are independent of ϕ . A scalar quantity $h(r, z, t)$ satisfying

$$(B_r, B_z) = (r^{-1} \partial h / \partial z, -r^{-1} \partial h / \partial r) \quad (2)$$

can then be used to represent the meridional components of $\mathbf{B}$. These components will vanish at $2m-1$ ($m = 1, 2, 3, 4 \dots \infty$) neutral points in the (r, z) plane, where m of these neutral points are of the O type, in the neighbourhood of which the field lines defined by (B_r, B_z) are elliptical in shape, and $m-1$ are of the X type, where the neighbouring field lines are hyperbolic in shape. Magnetic field lines in the (r, z) plane are parallel to contour lines of equal values of the scalar function h and the magnetic field strength is inversely proportional to the spacing of the contours. The neutral points of the O type are located at the positions of the local maxima in $|h(r, z, t)|$ and those of the X type at the cols or saddle points in $h(r, z, t)$.

Effects due to motional induction and Nernst–Ettinghausen e.m.f.s are represented by the second term on the right-hand side of equation (1). That these effects cannot prevent the ohmic decay (represented by the first term) of an axisymmetric magnetic field has now been proved in the most general case¹⁰, when $\mathbf{B}$ can be non-steady and σ and μ dependent on both position and time. The demonstration that axisymmetric magnetic fields always decay, though not in general monotonically, is effected by applying equation (1) to the global maximum $H(t)$ of $|h(r, z, t)|$, which occurs at the point $(\hat{r}(t), \hat{z}(t))$ (say). The main mathematical complication arising in the treatment of the general case is that $m, \hat{r}$ and $\hat{z}$ change discontinuously with time, but $H(t)$ decreases monotonically with time (in a piecewise differentiable manner) and this establishes the proof¹⁰.

The above results (which also apply to fields that are only topologically axisymmetric^{9,10}) must not be taken to imply that the magnetic field of a planet or star where it is usually observed—at or above the surface—must necessarily depart strongly in form from symmetry about some axis. The dipole component of any magnetic field configuration always dominates at great distances from the source of the field, simply because its rate of fall-off with distance from the source is slower than that of higher-order multipoles. If the dynamo region is confined to the deep interior, then non-dipolar features could be barely detectable at and above the surface even when they are pronounced well below the surface.

Another possible—though less probable—interpretation of a planetary or stellar magnetic field that above the surface appears to be highly symmetric about some axis is that the field is indeed axisymmetric everywhere, below as well as above the surface, but the e.m.f.s due to motional induction are (as a consequence of the axial symmetry) temporarily inoperative, the field having been caught by the observer in an ohmic decay phase. Recovery from this decay phase would, presumably, take place later, as soon as motions in the conducting fluid have reestablished suitable field asymmetries⁹, thereby switching the dynamo on again. But it would be practically impossible to see this decay and recovery in direct field observations, owing to the long time scales involved, many centuries at least for the Earth and much longer for the major planets Jupiter and Saturn and magnetic stars. On the other hand, such behaviour might manifest itself in the long-term variations in the Earth's magnetic field that have been inferred from the magnetism of certain sedimentary and igneous rocks and human artefacts (pottery, hearths). Thus,

palaeomagnetic and archaeomagnetic data might show evidence that departures from symmetry about some axis (not necessarily that of the Earth's rotation) are systematically less during the decay phase of a geomagnetic polarity 'reversal' or 'excursion' than during the growth or recovery phase and it will be of interest to analyse such data with this possibility in mind.

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First-order removal of particulate aluminium in oceanic surface layers

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Little is known about processes which remove particles with radii of $<5 \mu\text{m}$ from surface layers of the oceans although they influence the geochemical cycling of many substances. Agglomeration resulting from particle–particle interactions and/or incorporation into faecal pellets by organisms may accelerate the settling rate of such particles^{1,2}. We report here the vertical distribution of particulate aluminium in the Gulf Stream which is consistent with that expected if primary input were from the atmosphere and first-order removal occurred in the upper 100 m of the water column. We hypothesize that filter feeding, particularly by salps, may be responsible for calculated removal rates.

Samples were collected at $30^\circ 51.1' \text{N}$, $79^\circ 41.8' \text{W}$ in 525 m of water in late August 1979 using 30-l Teflon-coated GO-FLO (General Oceanics) bottles hung on Kevlar line. Preparation and analysis of these samples for particulate trace metal and particulate organic carbon analysis are described elsewhere³. Total dissolvable metals were determined by a procedure similar to those described in refs 4 and 5. Salinity, chlorophyll and nutrient concentrations were also determined. We were unable to measure temperature at this station but hydrographical data were obtained at nearby ($<10 \text{ km}$) stations 3 days later (L. P. Atkinson, unpublished results) and in July 1978 (ref. 6).

Nitrate, particulate organic carbon, particulate aluminium and chlorophyll concentrations are plotted in Fig. 1. Hydrographical data indicated the presence of a 30-m mixed layer with an intense pycnocline between 30 and 200 m. Nitrate concentrations were $<0.1 \mu\text{mol l}^{-1}$ from the surface to 100 m and then increased as a linear function of depth to 450 m. A deep chlorophyll maximum (DCM) was present near 100 m (refs 7–10). Particulate aluminium concentrations reached a minimum value at 100 m, coincident with the chlorophyll maximum, and then increased with depth, correlating well with nitrate concentration ($r^2 = 0.94$). (Large uncertainties in some of the aluminium data presented here reflect the fact that samples were necessarily processed in part outside a 'class 100' controlled environment. However, we believe the data are of sufficient precision and accuracy to support our conclusions.) The increase

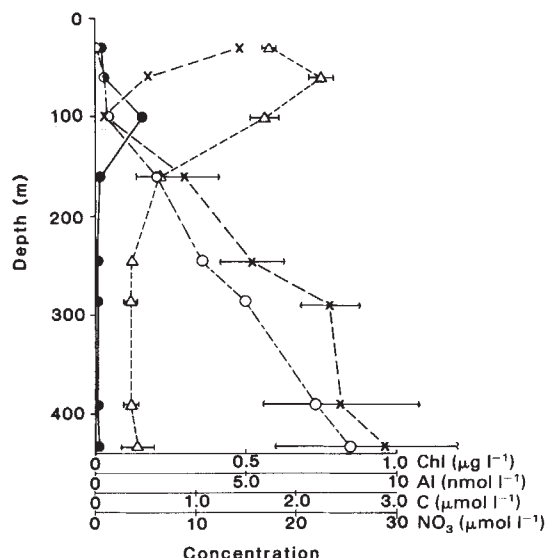


Fig. 1 Vertical profile of particulate aluminium (x), particulate organic carbon (Δ), nitrate (○) and chlorophyll (●). Error bars on carbon and aluminium values represent 1 s.d. Where error bars are absent, the uncertainties lie within the dimension of the symbol.

in particulate aluminium concentration with depth is not understood but may reflect resuspension and/or horizontal transport along isopycnals from the adjacent shelf.

We consider the particulate aluminium in the upper 100 m to be primarily of atmospheric origin because (1) the isopycnal at the base of the mixed layer (30 m) intersected the surface before reaching the shelf (L. P. Atkinson, unpublished results), (2) atmospherically deposited aluminium is essentially insoluble in seawater¹¹, and (3) the contribution to the concentration of particulate aluminium by adsorption of soluble aluminium is probably uniform with depth as the concentration of particulate and soluble forms of other particle-reactive elements, such as Cu, Zn, Ni and Cd, were homogeneous to 100 m. Mean concentrations of total dissolvable copper, zinc and nickel between 0 and 100 m were 1.03 ± 0.05 , 0.30 ± 0.05 and 2.3 ± 0.2 nmol l⁻¹ respectively, while cadmium increased slightly from <3 to 8 pmol l⁻¹ between 0 and 100 m. These concentrations are similar to those reported¹² for nutrient-depleted surface waters of the North Pacific. Mean particulate copper, zinc, nickel and cadmium concentrations over the same depth interval were 28 ± 6 , <16 , 18 ± 8 and 0.93 ± 0.13 pmol l⁻¹ respectively.

The residence time of particulate aluminium in the shallow mixed layer (0–30 m) may be calculated if atmospheric input is the primary source and steady-state conditions are assumed. An atmospheric flux of 5.8×10^{-2} nmol Al m⁻² s⁻¹, calculated from a geometric mean atmospheric concentration of 5.8 ± 1.8 nmol m⁻³ observed in southeastern Atlantic aerosols¹³ and deposition velocity of 1 cm s⁻¹ (ref. 14), was used to calculate a residence time of about 30 days in the mixed layer. The assumption of steady state is reasonable because intense stratification of the Gulf Stream occurs in this region by June¹⁵ and the geometric mean atmospheric aluminium concentrations of air reaching Bermuda from the area encompassing the upstream path of the Gulf Stream is similar to that observed in south-eastern Atlantic aerosols¹⁶.

Below the mixed layer, the exponential decrease in particulate aluminium with depth between 30 and 100 m is consistent with first-order removal by one or more mechanisms with an overall first-order removal constant of $k = 3.8 \times 10^{-2}$ m⁻¹ (Fig. 2).

A vertical model capable of explaining the observed vertical profile of aluminium assumes that: (1) atmospheric input is the only significant source of particulate aluminium in the 0–100-m water column, (2) atmospherically deposited aluminium settles in water with a constant mean velocity, V_s , until incorporated

into larger particles, (3) these large particles settle with much greater velocities and have much shorter residence times than small-particle aluminium in the 0–100-m depth interval, (4) steady-state conditions exist, (5) the net effect of horizontal advective and diffusive transport on the vertical profile of small-particle aluminium is negligible, and (6) the first-order removal rate is constant between 0 and 100 m.

The second assumption allows us to express the first-order constant k as a time-dependent constant k' defined by the following equation:

$$k' = kV_s \quad (1)$$

Under steady state, the total flux, which in this model is simply the sum of the small (F_s) and large (F_L) particle aluminium flux, is equal to the atmospheric flux (A). Small and large particle fluxes at any depth are given by

$$F_s = V_s[Al]_z \quad (2)$$

$$F_L = kV_sM_{0-z} \quad (3)$$

where $[Al]_z$ = concentration of small-particle aluminium at depth z = measured aluminium concentration at depth z , and M_{0-z} = total mass per m² of small-particle aluminium in the designated depth interval. The value of V_s may then be calculated from

$$V_s = A/([Al]_z + kM_{0-z}) \quad (4)$$

If $A = 5.0$ μmol m⁻² day⁻¹, $[Al]_z = 0.33$ μmol m⁻³ at $z = 100$, $k = 3.8 \times 10^{-2}$ m⁻¹ and $M_{0-z} = 263$ μmol m⁻² for a 0–100-m water column, then $V_s = 0.49$ m day⁻¹. A settling velocity of 0.49 m day⁻¹ is equivalent to the Stokes settling velocity of a spherical particle with a density of 2.5 g cm⁻³ and radius of 1.6 μm. This value compares favourably with the mass-median diameter of aluminium in marine aerosols^{14,16}.

From equation (1), $k' = 1.94 \times 10^{-2}$ day⁻¹, and the residence time of small-particle aluminium in the upper 100 m is $1/k'$ or 54 days with respect to first-order removal. That the first-order removal is due primarily to biological mechanisms and not to abiotic processes is supported by estimating the collision frequency of particles with radii of 1–5 μm. Following the procedures of Lal¹⁷, but adopting a collision efficiency factor of ~0.1 and a success factor of <1 (ref. 18), we estimate that the mean time between collisions is >2.8 yr. (Values used in equation (5) in ref. 17, after integration from $r_0 = 1$ μm to $r = 5$ μm, were $A = 45$ μg l⁻¹, $B = 10^4$ cm⁻¹ s⁻¹ and $b = 4$. The result was then multiplied by the product of the success and efficiency

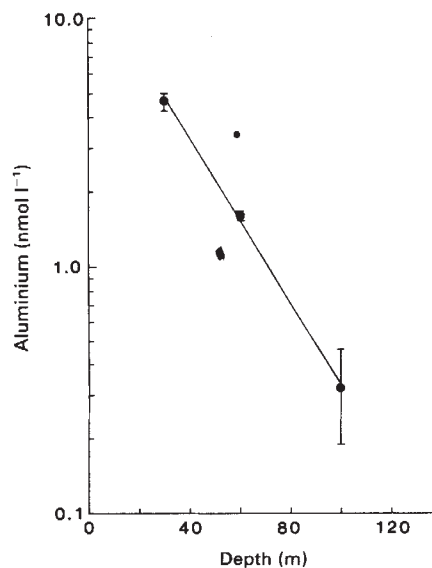


Fig. 2 log-linear plot of particulate aluminium versus depth between 30 and 100 m. Linear regression of log of particulate aluminium concentration against depth gave $m = -3.8 \times 10^{-2}$ m⁻¹, $r^2 = 0.996$.

probabilities.) The estimated time between collisions is considerably greater than the residence time of small-particle aluminium in the 0–100-m layer (0.15 yr).

The filtration rate of organisms required to satisfy observed first-order removal kinetics is $191 \text{ m}^{-3} \text{ day}^{-1}$, assuming 100% removal efficiency and a vertically homogeneous grazing rate between 0 and 100 m. Atmospherically deposited aluminium is primarily on particles with radii of $<2.5 \mu\text{m}$ ($>80\%$ of the aluminium in aerosols sampled in Enewetak¹⁴ and in Bermuda¹⁶ was on particles with apparent radii of $\leq 2 \mu\text{m}$), and filter feeders such as adult copepods cannot graze efficiently on particles of this size¹⁹. Larvaceans, naupliar and post-naupliar copepods, which are numerically abundant in the DCM region and are capable of grazing particles with $r < 2.5 \mu\text{m}$, constitute only a small fraction of zooplankton biomass ($\sim 20\%$ or $\sim 0.3 \text{ mg C m}^{-3}$) in the Northern Sargasso Sea²⁰. The clearance rate of these organisms, estimated by extrapolation of feeding experiments²¹ in which juvenile stages of *Eucalanus pileatus* were fed particles with $r < 2.5 \mu\text{m}$, is $< 1 \text{ ml l}^{-1} \text{ day}^{-1}$ at a biomass of $\sim 0.3 \text{ mg C m}^{-3}$. This filtering rate is clearly insufficient to influence significantly the vertical distribution of particulate aluminium.

The grazing activities of salps may explain the observed vertical distribution of aluminium. Salps (1) have filtration rates which may exceed $144 \text{ l m}^{-3} \text{ day}^{-1}$ (ref. 22), (2) efficiently remove particles with radii as small as $1 \mu\text{m}$ (ref. 23), (3) produce faecal material which settles with velocities $> 100 \text{ m per day}^{24}$, (4) have filtration rates which are independent of particle concentrations²², and (5) cover large distances while grazing²⁴. Unfortunately, fragmentary knowledge of the numerical abundance of salps in open ocean waters limits attempts to assess accurately the importance of these organisms with respect to first-order removal of fine particles in oceanic surface waters (L. Madin, personal communication). However, their potential importance in this regard and their concomitant influence on the biogeochemical cycling of trace elements and other substances warrant further study.

The data presented here show that the vertical distribution of particulate aluminium in highly stratified, oligotrophic open-ocean surface waters may help elucidate the processes affecting removal of fine particles from oceanic surface waters.

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What do the ants know about the rotation of the sky?

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Animals which use a celestial compass to navigate in a particular direction are able to compensate for the movement of the Sun^{1–3}. It has generally been assumed^{4–8} that this compensation is exact even though the rate of change of the position of the Sun's azimuth is not constant but varies according to the time of day, the date and the latitude. However, what can be deduced at best even from the most comprehensive studies of birds⁹, fishes¹⁰, spiders^{11,12} and bees^{13–16} is that the animals do not simply use the Sun's average rate of azimuth movement (15° h^{-1}). The scatter in most data and methodological shortcomings (for example, see ref. 17) prevent a more specific conclusion. A further possibility, supported by recent experiments with bees¹⁸, is that animals do not use long-term information about the rate of movement of the Sun's azimuth at all but just extrapolate from the most recently observed rate of movement. Here, we show that ants do indeed compensate for the variable rate of movement of the Sun's azimuth even if they have not seen the sky for several hours. Small but consistent errors further suggest that the ants might acquire their knowledge of the Sun's azimuth movement by interpolation between successive memorized positions of the azimuth.

For our experiments we used the genus *Cataglyphis*¹⁹, which consists of large, long-legged desert ants. Each ant forages individually^{20,21} and during its foray may cover distances of over 100 m from the nest. Unlike many other ant species²², *Cataglyphis* foragers do not navigate using scent trails but apply a dead reckoning strategy using celestial cues as a compass^{20,23–25}. At our field station in Maharès (Tunisia) more than 400 ants belonging to different colonies were trained to use different feeding stations positioned in different directions 15 to 30 m from the nest. When an ant arrived at a feeding station, it was transferred to a moist, light-proof glass flask, retained there for several hours and then released in a remote test area with which it was unfamiliar. After release it would set off in the home direction. The testing area, a hard sandy plain, was painted with a grid of fine white lines which allowed us to record the ant's path on a reduced scale for later statistical analysis. Furthermore, while homing, the ant was accompanied by a small vehicle that screened off all landmark cues and was loaded with optical equipment to shield or change the position of the Sun, or modify the pattern of polarized light in the sky (Fig. 1). As the person moving the vehicle was completely unaware of the ant's home direction as well as the rationale of the whole experiment, but was merely told to follow the ant, he was not biased towards any outcome of the experiment.

Due to the latitude and time of year, the Sun's rate of azimuth movement varied dramatically throughout the day: from 7.7° h^{-1} at dawn and dusk up to $56.1^\circ \text{ h}^{-1}$ at local noon. In the main set of experiments (Fig. 2) the ants were divided into two groups: one group (1) was trained in the morning when the Sun's azimuth moved slowly, and the other (2) was trained at noon when the Sun's azimuth moved fast. The hypothesis of linear extrapolation of the Sun's movement then predicts that ants released 2.7 to 5.9 h after training should (1) underestimate the movement of the Sun and thus deviate to the right from their home direction, and (2) overestimate the movement of the Sun and deviate to the left. However, no systematic deviation was observed. The ants' behaviour showed that they were compensating correctly for the movement of the Sun.

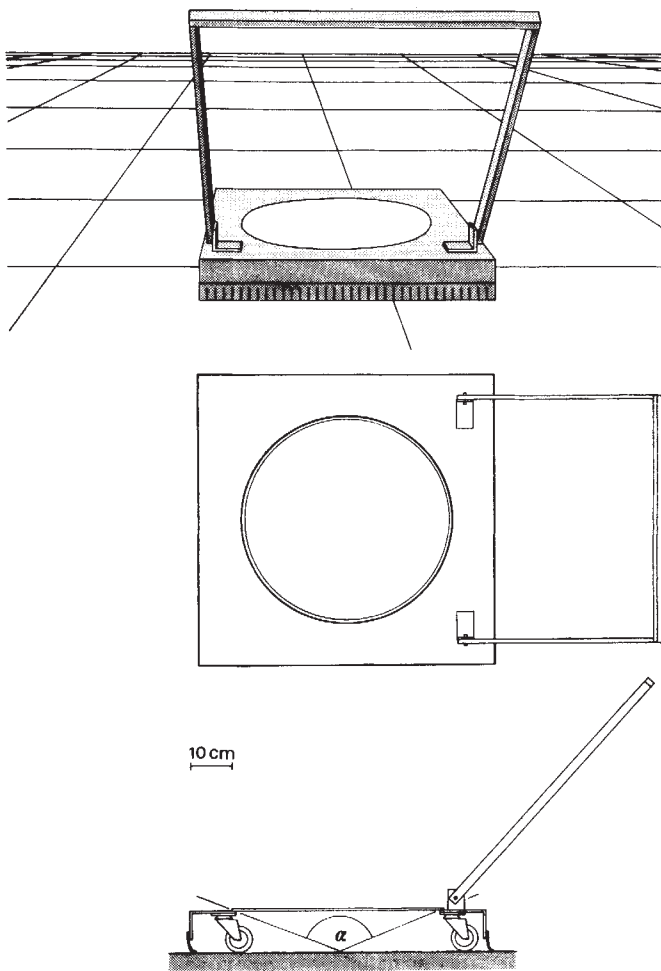


Fig. 1 Walking underneath an aluminium vehicle that was moved with the ant, *Cataglyphis* foragers viewed the sky through a circular window (diameter 134°) covered with UV-transparent Plexiglass (Roehm and Haas, no. 218). In all experiments performed later than 17.25 h the Sun was not visible to the ant. In various control experiments, the vehicle was equipped with polarizers, depolarizers, spectral cut off filters, or screens to shield the Sun. For further experimental details see refs 24, 25.

At least two control experiments are needed to substantiate this conclusion. First it has to be confirmed that the ants relied on celestial cues while navigating home. This was shown to be the case by screening off the Sun and depolarizing the light in the sky (or by using a long-wavelength cutoff filter to prevent stimulation of the UV receptors necessary for the detection of polarized light²⁴). In these conditions the ants were unable to choose any compass direction at all ($n = 54$). Furthermore, if a strongly polarized horizontal e -vector (marking the antisolar meridian) was displayed to the ants, for example, 90° off the true antisolar meridian in the sky, the ants deviated from their home direction by about the same angular amount when tested at a later time in the day ($n = 16$). Second, there is the possibility that the ants did not compensate for the Sun's movement at all, but inferred their home direction just from celestial cues present at the time of release. In principle, they could do so by observing the rotation of the pattern of polarized light about the north pole of the sky. However, they were well oriented even if they could not observe the pattern of polarized light but only the Sun ($n > 200$). Furthermore, foraging bees in which the internal clock had been time-shifted¹⁵ or which had been trained at one longitude then tested at another (but at the same latitude)¹³, did not orient in their true home direction as one would expect if they had determined true north by observing the rotation of the celestial

sphere. Similar experiments performed with amphipods^{26,27} confirm this conclusion.

Another series of experiments was designed to determine the fine structure of the ant's internal representation of the Sun-azimuth curve. In these experiments the ants were kept in the dark for only 1 h, thus they were tested exactly 1 h after training. Furthermore, the experiments were done at a time of the year when the daily variation of the Sun's rate of azimuth movement was greater (ranging from 7.3° h^{-1} at sunrise to $68.9^\circ \text{ h}^{-1}$ at noon) than in the experiments described in Fig. 2. As shown in Fig. 3, the ants underestimate the rate of movement of the Sun's azimuth when it is high, and overestimate this rate of movement

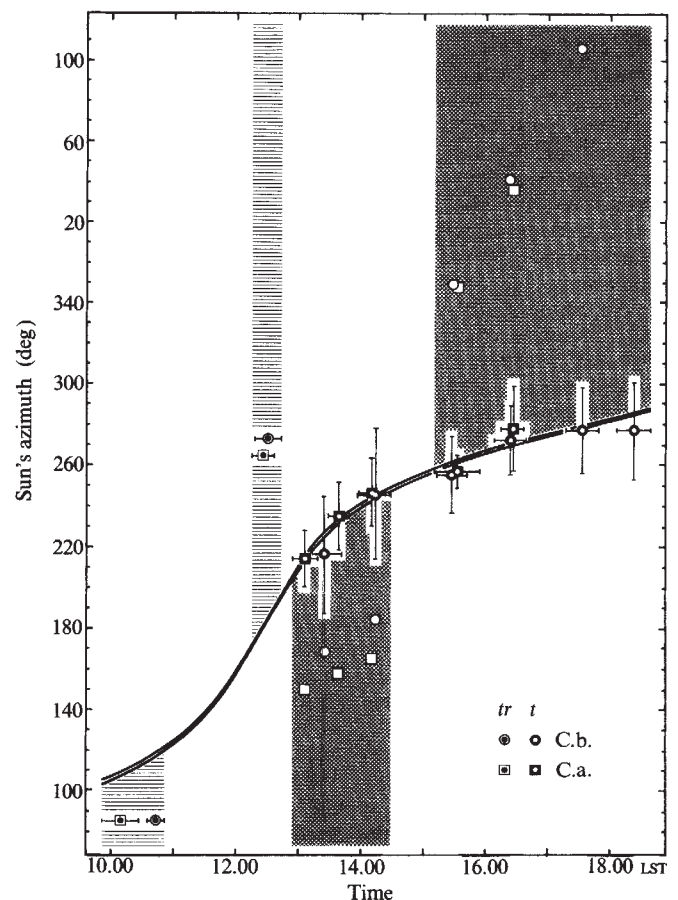


Fig. 2 This shows how ants compensate for the movement of the celestial sphere. The data points (t) indicate where the ants expect the solar meridian to be when tested 2.7 to 5.9 h after they have last seen the sky during training (tr). The training and testing times are indicated by light and heavy shading, respectively. The two solid lines include the positions of the Sun's azimuth (solar meridian) for the period 23–29 July at Maharrès, Tunisia (latitude 34.58° N , local noon at 12.24 h local standard time, LST). If the ants were informed correctly about the Sun's movement in azimuth, that is, if they followed the correct home direction, the data points would lie on the Sun-azimuth curve. Data points are means $\pm$ s.d.; s.d. = $\sqrt{2(1-r)} 180/\pi$ (deg), where r is the length of the mean vector. If the ants underestimated (or overestimated) the movement of the Sun, they would deviate to the right (left) from their home direction. Consequently, the data points would lie below (or above) the Sun-azimuth curve. In both morning and noon experiments (indicated by shading below and above the Sun-azimuth curves, respectively) the ants expect the Sun in about the right position. If they had linearly extrapolated the Sun's rate of movement from the time when they had last seen the sky, their walking directions would have deviated considerably from true home (see open symbols within the shaded areas). In each case, the latter hypothesis can be rejected ($P < 0.001$), as can the hypothesis that the ants refer to a mean rate of the Sun's movement, that is, to 15° h^{-1} ($P < 0.001$, Stephens test²⁸). C.a., *Cataglyphis albicans*-A³⁰; C.b., *Cataglyphis bicolor*; $n = 153$.

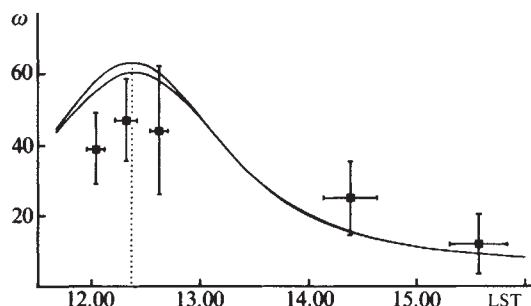


Fig. 3 Compensation errors made by the ants *C. bicolor*, in accounting for the daily movement of the Sun's azimuth. The solid lines indicate the change of the Sun's azimuth position per hour, ω (deg h^{-1}), during the time of observation. Broken line, local noon. The data points (means $\pm$ s.d.) represent the ants' estimates of the Sun's change in azimuth position during the time of arrest (1 h) as deduced from the homeward courses of the ants. These estimates differ significantly from theoretical values ($P < 0.01$) except those values at 15.34 h ($n = 67$).

when it is low. Thus, their internal representation of the Sun's rate of azimuth movement is a slightly smoothed version of the true Sun-azimuth curve. This effect was not shown by previous experiments (Fig. 2), in which the ants were kept in the dark for several hours. There, the ants trained in the morning and tested at noon overcompensated in the first part of their dark period and undercompensated in the second part, so that finally both effects roughly cancelled out. Similarly, the ants that were trained at noon and tested in the afternoon first undercompensated and then overcompensated.

How do ants gain information about the movement of the Sun? Certainly, insects, unlike astronomers, do not perform spherical trigonometry in the sky. It is possible that they infer the movement of the Sun's azimuth, that is, the movement of the solar or antisolar meridian marking the symmetry line of the pattern of polarized light, relative to their known landmark map. Each *Cataglyphis* forager leaves the entrance of its underground nest in a particular direction over periods of several days or weeks. As this orientation is governed by the landmark panorama around the nest, this panorama is likely to be the system of reference used by the insect to measure the different positions of the Sun's azimuth at different times of the day. In addition, if the ant is able to memorize different azimuth positions of the Sun for different times of the day, it could use this information to assemble an internal model of the daily azimuth-movement of the Sun, that is, interpolate between successive memorized positions by assuming that the intermediate angular change of the Sun's azimuth is proportional to time. This hypothesis is supported by the type of compensation error made by the ants (Fig. 3): they underestimate the highest rates of movement of the Sun's azimuth and overestimate the lower rates. Similar experiments²⁸ with ants kept for several hours in the dark and released at night (when they were tested in the presence of an artificial light source) or on the following day demonstrated that the ant's internal system of compensation is not restricted to the visible part of the Sun's arc but performs a 24-h cycle very similar to that of the Sun's azimuth.

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Epidermal feet in insect morphogenesis

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In many insects the larval cuticle pattern changes at metamorphosis to one of transverse ripples, often with a shortening of the segments (for example, *Rhodnius*, Hemiptera¹; *Calpodes*, Lepidoptera; *Tenebrio*, Coleoptera²). The orientation of these ripples in *Rhodnius* was used to discover a segmentally repeating gradient of positional information³. Since then there has been much work on the gradient^{4–6} but little on the mechanism by which an epidermal cell controls the cuticle pattern that allows us to read its orientation in the gradient. We report here that in the larval/pupal transformation of *Calpodes* and other insects, the epidermis develops basal cytoskeletal extensions or feet, oriented in the direction of the gradient at right angles to the ripples. Their contraction coincides with segment shortening and transverse ripple formation. Epithelial cells of the integument are not always packed together like polygonal paving stones as they are usually depicted, but may relate to one another by cytoskeletal extensions of a kind that are more usually reported from separate moving cells in tissue culture. Such epidermal feet may have general relevance in morphogenesis.

Conventional whole-mount preparations of the integument for light microscopy do not usually resolve fine processes such as the feet, although they are clearly visible in thin sections by electron microscopy. We have developed a procedure to show the feet that gives an effect as if thousands of cells randomly arranged in the epithelium had each been injected with a lead salt visualized as black lead sulphide⁷. The procedure depends on the fact that after brief glutaraldehyde fixation, tannic acid only penetrates some cells where it mordants lead ions. Individual cells visualized in this manner appear as if they are separate in a tissue culture although they are part of a closely packed epithelium (Figs 1, 2). In mid-fifth instar *Calpodes* the feet form an interdigitating basal web of branched extensions joined by hemidesmosomes to the basal lamina and by gap junctions to one another. At the time of their greatest extension they are predominantly axially oriented and in some areas individual feet can be followed for as far as four cell lengths. In at least some cells the feet are not only axially oriented but polarized, with the anterior extensions being more finely divided than those pointing posteriorly (Fig. 2). The polarity of the feet is presumably a particular aspect of the orientation of the cytoskeleton which may itself be related to the arrangement of basal bodies, as has been shown for the formation of microtrichia in

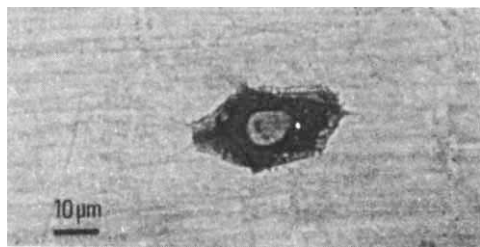


Fig. 1 The shape of a cell in the epidermis at E+64 h before the initiation of feet. Whole mount of the dorsal integument prepared by the following procedure. Larvae were decapitated and the haemolymph allowed to drain. They were then inflated with cold primary fixative consisting of 5 g digallic acid neutralized with NaOH (Mallinkrodt, Tannic Acid AR powder, 1764) and 5 g pyrogallol in 100 ml 5% glutaraldehyde in 0.05 M phosphate buffer at pH 7.6. After 5 min the larvae were cut up in cold fixative and left for 2 h at 0–4 °C. Cut pieces of integument were then cleaned of muscle and fat body and left for hours to days in the above mixture without glutaraldehyde. Cleaned pieces were brought to room temperature and transferred successively to 0.2 M sodium cacodylate buffer at pH 7.4; 0.1 M sodium cacodylate buffer at pH 5.4; stock aspartate at pH 5.4 consisting of 0.988 g L-aspartic acid in 250 ml distilled water + 1 M KOH to dissolve and bring pH to 5.4; Two changes of lead staining solution consisting of 0.0666 g lead nitrate added to 10 ml of the stock aspartate solution having the pH adjusted to 5.4 with KOH for 1–2 h on a gentle rotary shaker (lead acetate can also be used for the staining solution); Stock aspartate buffer pH 5.4 without lead; two changes in 0.1 M cacodylate at pH 5.4. The lead was then visualized by 10 min exposure to 5–10% yellow ammonium sulphide before dehydration and mounting. Additional contrast can be obtained if needed by immersion in 1% OsO₄.

Sarcophaga epidermis⁸. The terminations of the feet may be as fine as 100 nm in diameter and can only be resolved by electron microscopy. They contain bundles of microtubules and microfilaments that are continuous with the cytoskeleton of the cell body. Structurally they are identical with the epidermal strands described from the intersegmental boundaries of *Rhodnius* by Wigglesworth⁹ but their relationship to the epidermal extensions controlling tracheole distribution^{10,11} is uncertain.

There are three phases in the life of the feet: initiation, extension and shortening (Fig. 3). The timing of each phase coincides with changes in haemolymph ecdysteroid titre¹², suggesting that development of the feet may be hormonally controlled. There are no feet at larval moults or early in the stadium (Fig. 1). The feet begin their growth in the fifth and last larval stadium with the ecdysteroid peak signalling epidermal commitment for pupation (↓↑↓) (that is, after this time high titres of moulting hormone cause a pupal rather than a larval moult). The feet continue to grow during the ensuing intermoult phase of low but measurable and steadily rising haemolymph ecdysteroids (Fig. 2). The third phase of foot shortening, segment shortening and a decrease in cell area as the cells become columnar, coincides with the prepupal hormone peak.

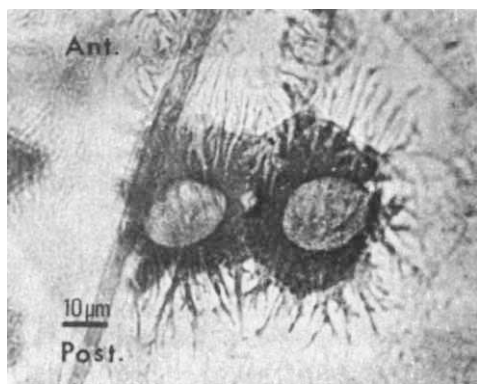


Fig. 2 Mid-instar larvae committed to pupation have basal processes or feet. In at least some cells the feet are polarized with very fine dendrites pointing anteriorly (Ant) and coarser structures projecting towards the posterior (Post) margin of the segment. Dorsolateral cells from the abdomen of a mid-instar larva. Preparation as in Fig. 2.

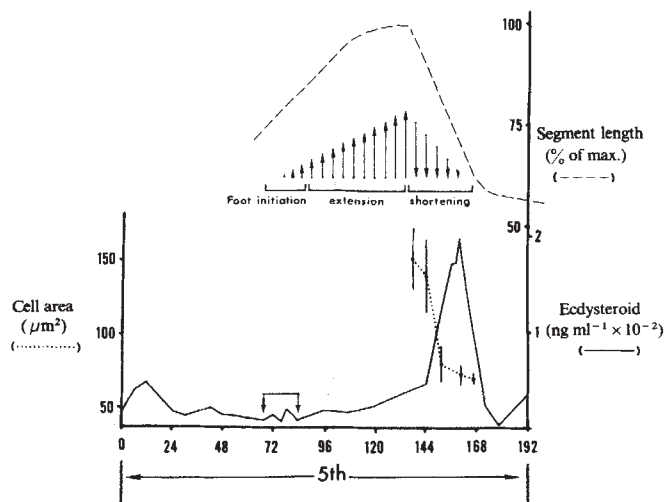


Fig. 3 Changes in the epidermal feet (↑↑↓) in relation to segment shape, cell area and haemolymph ecdysteroid titre¹¹ (—) during the fifth stadium of *Calpodes ethlius* (Lepidoptera, Hesperidae). Feet are absent early in the stadium. They begin to form after the commitment peak of ecdysteroid (↓↑↓) for pupation and grow during the intermoult period of low but measurable hormone from E+84–134 h. They shorten at the beginning of the prepupal peak as the cell area decreases (....) and the segment itself shortens (---).

Artificially administered hormone mimicked the growth that coincided with these natural hormonal changes. Growth of the feet in isolated abdomens was enhanced by 1–3-day exposures to low titres (100–200 ng per larva) (Fig. 4), while high titres (2 μg per larva) caused the feet to contract as the cells became columnar and the segment shortened. The cytoskeleton of epidermal cells *in situ* thus responds to ecdysteroids like *Drosophila* cells in tissue culture^{13,14}.

The timing of contraction of the feet coincides with shortening of the larval segment to its pupal form, suggesting that the feet are necessary for this shape change. To confirm this, isolated larvae that were committed to make pupae at the next moult were caused to lose their feet either by the injection of 2 μg of colchicine or by storing them for longer than 8 days. Such abdomens when forced to moult by the injection of 20-hydroxyecdysone, formed pupae that had larval segment proportions and cuticle without transverse ripples. We conclude that feet are necessary for segment shortening and cuticle ripple formation at metamorphosis.

The epidermal feet may have general relevance for insect morphogenesis¹⁵, performing the same kind of role as the filopodia that extend across the archenteron of sea urchin embryos where they search for the appropriate surface before contracting to cause gastrulation¹⁶. They may often have been overlooked because of the difficulty of demonstrating the outlines of single cells that are united in epithelia. In *Calpodes*, in addition to their association with segment shortening, the feet

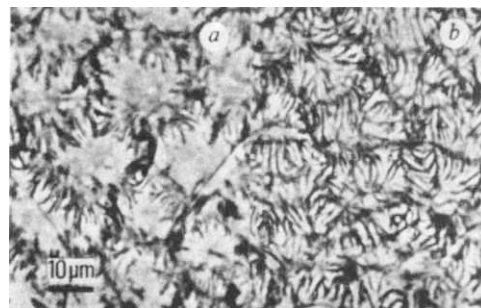


Fig. 4 a, The feet shortly after their initiation. b, One day after the administration of 100 ng 20-hydroxyecdysone to an isolated abdomen. Phase contrast of dorsolateral integument with lightly stained feet as in Fig. 2.

are arranged around the dying prolegs, so that contraction may close the gap that is left in the integument. The feet around muscle insertions are often orientated towards them, suggesting that they may be concerned in their repositioning². In *Drosophila* larvae the feet have an axial orientation appropriate for puparium formation and in the dorsal segments of *Rhodnius* we have found feet that may be the immediate cause of segment shortening and cuticle ripple formation.

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Defective up-regulation of the androgen receptor in human androgen insensitivity

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Genital skin fibroblast strains have helped to identify heritable defects of the androgen-receptor system that cause androgen insensitivity (AI). Three quantitative types have been defined: receptor-negative¹ (no or barely measurable androgen-receptor activity); receptor-deficient^{2,3} (easily measurable but less than normal); and receptor-positive^{4,5} (activity in the normal range: 15–50 fmol per mg protein). As the clinical extent of AI cannot be correlated with receptor activity, various qualitative receptor defects have been sought and found, including receptor thermolability⁶ and an increased rate of dissociation of androgen-receptor complexes^{7–9}. We report here that normal human genital skin fibroblasts preincubated with 5 α -dihydrotestosterone (DHT) for 19 h at 37 °C have twice as much DHT-receptor activity as unexposed replicates (basal). The increase is blocked by cycloheximide; thus it is a form of 'up-regulation' that is probably due to androgen-induced receptor synthesis. After DHT preincubation the fibroblasts from three subjects with three types of receptor-positive AI did not augment their basal activities. Such an aberrant response may underlie some cases of receptor-positive, steroid-resistant breast cancer in women¹⁰.

In most laboratories that investigate constitutional AI at the cellular level, whole cell androgen-receptor activity is routinely assayed by preincubating confluent fibroblast monolayers in serum-free medium (SFM) overnight and then exposing them to ³H-DHT in SFM at 37 °C for about 60 min; that is, until saturation of the specific (high-affinity) androgen receptor binding sites occurs^{2–4}.

Our study was designed to determine whether normal genital skin fibroblasts could adapt to the presence of androgen in their

medium by increasing their androgen-receptor activity; and to assess whether fibroblasts from receptor-positive androgen-insensitive subjects could adapt in the same way. We determined this in two ways.

Table 1 presents the data obtained when the fibroblasts were fed SFM containing 3 nM ³H-DHT 19 h before initiating a standard 1-h assay by refeeding the monolayers with a fresh batch of SFM containing 3 nM ³H-DHT. Fibroblast strains derived from foreskin and labium majus skin behaved identically, there being a doubling in DHT-receptor activity, and the variation within each group was independent of the chronological age of the donors from whom the primary skin explants were obtained, or the *in vitro* age (mean population doubling level) of the strains at the time of assay.

Table 2 presents the data obtained when the fibroblasts were preincubated with 10 nM radioinert DHT and then had their DHT-receptor activities determined by a 2-h ³H-DHT exchange assay. The degree of augmentation in the control strains was the same as that observed in Table 1, indicating: (1) that the degree of augmentation is independent of the initial preincubation DHT concentration in the range of 3–10 nM; and (2) that the augmented DHT-receptor activity is qualitatively the same as the basal activity that is measured in the routine 1-h assay without DHT preincubation. The latter indication is supported by the facts that the ³H-DHT-receptor complexes formed in the basal and augmentation conditions have the same rate constant of dissociation ($6 \pm 0.3 \times 10^{-3} \text{ min}^{-1}$; $\bar{x} \pm \text{s.e.m.}$); and that augmentation is blocked by the protein synthesis inhibitor, cycloheximide. We do not yet know whether cycloheximide is blocking an increased rate of receptor synthesis or causing a decreased rate of receptor degradation, or doing both.

In contrast to the adaptive behaviour of the control strains, fibroblasts from three subjects with receptor-positive AI failed to augment their DHT-receptor activities after DHT preincubation (Table 1).

Subject TC was born with ambiguous external genitalia and reared as a male with corrective surgery. He has one affected sibling and four other maternally related affected relatives, all of whom have been reared as females. He thus has a form of partial AI that is probably determined by a mutation at the X-linked locus that codes for the androgen receptor in man¹¹, mouse¹² and, almost certainly, all mammals¹³. We have previously shown that the fibroblasts of TC form a normal number of androgen-receptor complexes during a 1-h assay at 37 °C and that these complexes are not only thermolabile when assayed at 42 °C, but also dissociate three times faster than normal^{7–9} at 37 °C.

Subject 14679 was also born with ambiguous external genitalia. He has no other affected relatives and his capacity for testosterone biosynthesis is normal under human chorionic gonadotropin (hCG) stimulation. His DHT-receptor complexes show neither the thermolability nor the dissociative defect seen in TC. Thus, the failure of his fibroblasts to up-regulate represents the only *in vitro* marker available for this type of partial AI. If his mutation is X linked as well, this marker will be useful for determining whether his mother (and perhaps her female relatives) are heterozygous carriers by searching for the cellular mosaicism among their fibroblasts that is expected from random X-chromosome inactivation (the Mary Lyon hypothesis).

The fact that TC shares only his up-regulatory defect with 14679 indicates that different mutations are responsible for their respective states of partial AI, which are clinically very similar.

Subjects KI, RE, NH and JR have the classical clinical phenotype of complete AI (formerly 'complete testicular feminization'). KI has no affected relatives; her testosterone biosynthetic capacity was ascertained to be normal under hCG stimulation. Her fibroblasts form normal amounts of DHT-receptor complexes at 37 °C. These complexes are more thermolabile than those of TC when assayed at 42 °C, but they dissociate at an equally abnormal rate^{7–9}.

Table 1 Specific ^3H -DHT-receptor activity after no or 19-h preincubation with 3 nM ^3H -DHT in fibroblasts from 10 control subjects and 6 with four different types of androgen resistance

Type	Strain	^3H -DHT-receptor activity (fmol per mg protein)		
		0	19 (cycloheximide)	19/0 No.; range)
Control	XAF*	23.6	45.6	1.9 ; 1.6–2.6)
		40	65 (28)	
	SISF	23.5	53.5	2.3 (2; 1.9–2.8)
		30	59 (21)	
	PUF	15	38	2.5
	LCF	15	31 (9)	2.0
	F70478	21.5	56.5	2.6 (2; 2.5–2.7)
	F80044	19.5	50	2.5 (2; 2.3–2.8)
	F80055	27	53	1.9
	L12	30.5	59	1.9 (2; 1.9–1.9)
	L26	23	52.5	2.3 (2; 1.9–2.7)
	L32	12	25.5	2.1 (2; 2.0–2.2)
		14	31 (14)	
Receptor-positive partial AI				
Type 1, TCF		29	24	0.87 (4; 0.63–1.0)†
Type 2, F14679		36.7	33.5	0.91 (4; 0.65–1.05)†
Receptor-positive complete AI				
KIL		24	16	0.67 (2; 0.60–0.75)†
Receptor-negative complete AI				
REL		<3	<2	— (2)
NHL		<2	<2	— (2)
JRL		<2	<2	— (2)

Genital skin fibroblast monolayers were grown to confluence in Eagle's minimal essential medium made with Earle's salts and supplemented with 2 mM glutamine, 1 mM pyruvate, $1 \times$ non-essential amino acids and an equal mixture of newborn and fetal calf serum (10%, v/v). Nineteen h before assay, replicate monolayers were washed free of growth medium, divided into three groups and fed: medium I, SFM (additionally buffered to pH 7.4 with 15 mM HEPES, 2N-hydroxyethylpiperazine-2-N'-ethanesulphonic acid); medium II, SFM with 3 nM [1, 2, 4, 5, 7- ^3H]5 α -dihydrotestosterone (130 Ci mmol $^{-1}$; NEN); or medium III, medium II plus 0.6 μM radioinert DHT. At the time of assay, triplicate monolayers in group I (0 h DHT preincubation) were washed and fed either medium II (to measure 'total' binding) or medium III (to measure 'nonspecific' binding). Likewise, triplicate monolayers in groups II and III were refed media II and III respectively to measure total and nonspecific binding after 19 h DHT preincubation. In some experiments, 2 μM cycloheximide (Sigma) was present in replicate cultures during both the preincubation and assay periods. The assay was terminated 1 h later and thereafter the cells were handled⁶ and sampled for protein and radioactivity⁵ as described in detail previously. DHT-receptor activity is stable in cells preincubated in the absence of androgen for at least 72 h before assay^{5,14,15}. Not more than 25% of the initial 3 nM ^3H -DHT is metabolized during a 1-h assay at 37°C (refs 6, 14). The equilibrium dissociation constant (K_d) of the androgen-receptor activity in genital skin fibroblasts is $\sim 1 \text{ nM}$ ^{1,3,5}. The individual results on replicate monolayers in both the total and nonspecific groups seldom differ from their respective means by >10%. Nonspecific binding (receptor activity) is seldom >10% of total binding. The control strains were derived from pieces of foreskin or labium majus skin donated by normal volunteers with informed consent according to protocols approved by the Ethics Committee of the Hospital. Basic information on the strains derived from JR¹⁶, RE, NH and KI⁵ has been provided previously, as have the data defining the thermal and dissociative androgen-receptor defects in the fibroblasts of KI and TC, and the dissociative behaviour of normal androgen-receptor complexes^{7–9}.

* Codes containing the letter F or L indicate strains originating from foreskin or labium majus skin respectively.

† Each of the 10 experiments performed on the strains from TC, 14679 and KI included at least one from a control subject. In none of these experiments did the activity of a mutant strain after DHT preincubation exceed the value measured after no preincubation.

The fact that TC and KI share their up-regulatory defect with 14679, but not their thermolability and dissociative defects, indicates that the former defect is functionally independent of the latter two. Indeed, assuming that there is a structural domain on the receptor protein that is essential for the androgen-receptor complex to interact with chromatin as a signal for up-regulation, we speculate that the mutation of 14679 affects that domain specifically, while those of TC and KI affect both the former and the hormone-binding site. Alternatively, the mutation of 14679 may be at the chromatin acceptor site, or at some more distal (post-receptor) step in the molecular apparatus underlying target cell responsiveness to androgen.

RE, NH and JR all have positive family histories typical of X-linked recessive inheritance. Their fibroblasts have no or severely deficient DHT-receptor activity, which is not augmented by DHT preincubation. Thus, if their respective mutations are of the regulatory (rather than structural) type, then none is vulnerable to up-regulation as defined herein.

We do not know the extent to which the up-regulation defect (we have defined *in vitro*) is responsible for the clinical phenotypes of subjects TC and KI, nor whether it can, by itself, explain the phenotype of subject 14679. For example, in the case of 14679, it is simple to imagine that normal up-regulation is developmentally important, if one accepts that cyclical or other physiological fluctuations in the level or availability of free DHT may confer an adaptive advantage on target cells that are able to increase their androgen-receptor activity in response to DHT. However, as we have not studied the behaviour of these mutant cell strains at every level of the androgen response system, the answers to these questions remain incomplete.

Finally, we can interpret our data on the three subjects with receptor-positive AI as follows: in the absence of androgen, the

balance of processes that regulates the synthesis and degradation of the androgen receptor protein in their genital skin fibroblasts is normal; in the presence of androgen, the balance becomes abnormal.

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Table 2 Specific ^3H -DHT-binding activity measured by a 2-h ^3H -DHT exchange assay after a 1- or 20-h preincubation with 10 nM radioinert DHT in fibroblasts from three control subjects and one with presumptive androgen insensitivity

Strain	^3H -DHT-receptor activity (fmol per mg protein)				
	Preincubation with DHT (h)	0	1*	20	20†/0
SISF	30	15	32	64	2.1
L26	36	20	34	61	1.7
F1778	30	18	35	58	1.9
F60979‡	20	11	27	49	2.4

Replicate monolayers were divided into three groups and preincubated as follows: (1) SFM alone for 20 h; (2) SFM for 19 h, then SFM with 10 nM radioinert DHT for 1 h; (3) SFM with 10 nM DHT for 19 h, then the same medium for 1 h. The preincubation period was terminated by washing all the monolayers extensively before initiating the 2-h exchange assay with 3 nM ^3H -DHT. All subsequent steps were performed as described in Table 1 legend.

* The values observed after preincubation with radioinert DHT for 1 h are about one-half those observed after no DHT preincubation. This confirms the fact⁹ that DHT-receptor complexes dissociate with a $t_{1/2} = 2$ h, a value equivalent to the rate constant of dissociation $k_{-1} = 6 \pm 0.3 \times 10^{-3} \text{ min}^{-1}$.

† Values calculated from the values observed after 0, 1 and 20 h preincubation with radioinert DHT on the basis that the augmented level of DHT-receptor complexes also has a $t_{1/2} = 2$ h. Thus $20^\dagger = 20(0/1^*)$.

‡ This strain, derived from a subject with a clinical diagnosis of AI, up-regulates normally; thus we still do not have an *in vitro* marker of the molecular defect that is responsible for his particular type of androgen-resistant state.

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Autoregulation of androgen production in a primary culture of rat testicular cells

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Studies on hypophysectomized rats have suggested that testicular androgen production may be locally autoregulated by an ultra-short loop negative feedback mechanism^{1,2}. Conclusive demonstration of this phenomenon *in vitro* has been hampered by lack of a functional testicular cell culture and the use of native androgens which are subject to extensive metabolism and interfere with measurements of endogenous androgens. We have now used a recently developed primary culture of rat testicular cells³ and synthetic steroids, and report that a synthetic androgen (R1881)⁴ decreases, whereas a synthetic anti-androgen (cyproterone acetate)⁵ increases, the gonadotropin-stimulated accumulation of testosterone *in vitro*. Furthermore, cyproterone acetate antagonized the inhibitory effect of R1881 on the accumulation of testosterone. In contrast, R5020 (ref. 6), a highly specific and metabolically stable progestin, did not affect the gonadotropin-stimulated accumulation of testosterone. These findings are the first *in vitro* demonstration of local autoregulation of testicular androgen production and suggest that the intratesticular negative feedback action of androgens may be mediated by specific testicular androgen receptors.

The direct effects of R1881 and cyproterone acetate on the human chorionic gonadotropin (HCG)-stimulated accumulation of testosterone by testicular cells were investigated *in vitro*³. Treatment with HCG (10 ng ml⁻¹) resulted in a 40-fold increase in the accumulation of testosterone over untreated controls (Fig. 1), whereas treatment with R1881 or cyproterone acetate by themselves had no effect on the basal accumulation of testosterone (Table 1). Concomitant treatment with increasing concentrations (10⁻⁹–10⁻⁵ M) of R1881 led to dose-dependent decreases (up to 74%) in the HCG-stimulated accumulation of testosterone with a median effective dose (ED₅₀) of 5.6 ± 0.3 × 10⁻⁶ M. In contrast, concomitant treatment with increasing concentrations (10⁻⁹–10⁻⁵ M) of cyproterone acetate resulted in a biphasic effect, whereby lower doses (10⁻⁹–10⁻⁶ M) increased the HCG-stimulated accumulation of testosterone. The ED₅₀ for the stimulatory effect of cyproterone acetate was 2.8 ± 0.2 × 10⁻⁷ M. Higher doses (10⁻⁶–10⁻⁵ M) of cyproterone acetate were less effective in increasing the HCG-stimulated accumulation of testosterone, although 10⁻⁵ M doses still caused increases of 59%. In addition, treatment with cyproterone

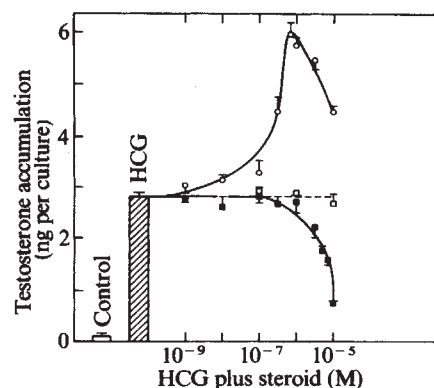


Fig. 1 Effects of treatment with R1881 (■), cyproterone acetate (○) or R5020 (□) on the HCG-stimulated accumulation of testosterone by rat testicular cells. Cells (2 × 10⁶ viable cells per dish) were obtained from adult (50–70-day-old) hypophysectomized male rats and cultured without treatment for 10 days, media being replaced every 2 days³. Leydig cell representation on day 10 of culture (as determined by histochemical staining for 3β-hydroxysteroid dehydrogenase) ranges from 10 to 30% of the total cell number. At the end of 10 days, the cells were reincubated for 2 additional days in the presence or absence of HCG (CR-121, 13,450 IU mg⁻¹) and increasing concentrations (10⁻⁹–10⁻⁵ M) of R1881 (methyltrienolone, Roussel-Uclaf), cyproterone acetate [17α-acetyloxy-6-chloro-1,2-dihydro-1(β,2β)-3'H-cyclopropa-(1,2)pregna-1,4,6-triene-3,20-dione] (Schering) or R5020 (promegestone, Roussel-Uclaf). Media testosterone content was measured by radioimmunoassay. Data points represent mean ± s.e., n = 4. None of the test compounds used cross-reacted with the testosterone radioimmunoassay (data not shown). Measurements were further validated by the demonstration of comparable testosterone levels in representative unextracted, ether-extracted and HPLC samples. Testosterone (ng ml⁻¹) measurements in unextracted, ether-extracted and HPLC samples were 6.22, 6.01 and 6.1 for cells treated with HCG, 9.38, 9.51 and 9.76 for cells treated with HCG + cyproterone acetate (10⁻⁶ M) and 3.24, 3.05 and 3.14 for cells treated with HCG + R1881 (10⁻⁵ M). HPLC was performed with an ALTEX, reverse-phase column, using acetonitrile/water (40:60) as the mobile phase. Similar results were obtained using celite chromatography as previously described¹⁹.

acetate partially blocked the R1881 (5.6 × 10⁻⁶ M)-induced inhibition of the HCG-stimulated accumulation of testosterone as indicated by 2.36- and 0.32-fold increases in the accumulation of testosterone by cells treated with 10⁻⁶ and 10⁻⁵ M of cyproterone acetate (Table 1). In direct contrast to the modulatory effects of the androgen and antiandrogen, concomitant treatment with increasing concentrations of R5020 (10⁻⁹–10⁻⁵ M) had no effect on the HCG-stimulated accumulation of testosterone.

Additional studies were performed to examine the direct effects of R1881 and cyproterone acetate on the choleragen and dibutyryl [(Bu)₂-] cyclic AMP-stimulated accumulation of testosterone (Table 2). Treatment of testicular cells with choleragen (10 ng ml⁻¹) and (Bu)₂-cyclic AMP (0.5 mg ml⁻¹) led to 60- and 70-fold increases in the accumulation of testosterone, respectively. Concomitant treatment with R1881 (10⁻⁵ M) inhibited both the choleragen-stimulated accumulation of testosterone (by 62%) and the (Bu)₂-cyclic AMP effect (by 97%). In contrast, concomitant treatment with cyproterone acetate (10⁻⁶ M) increased the choleragen-stimulated accumulation of testosterone by 133%, whereas 3 × 10⁻⁶ M of cyproterone acetate doubled the (Bu)₂-cyclic AMP effect. As in the case of HCG, a higher dose (10⁻⁵ M) of cyproterone acetate was less effective in increasing the choleragen- and (Bu)₂-cyclic AMP-stimulated accumulation of testosterone.

The cellular mechanisms whereby androgens regulate their own production are unknown. However, the ability of R1881 and of cyproterone acetate to modulate the (Bu)₂-cyclic AMP-stimulated accumulation of testosterone suggests that their effect is exerted, at least in part, distal to the generation of cyclic AMP. Although the exact site of autoregulation remains

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Table 1 Effects of treatment with cyproterone acetate (CPA) on R1881-induced inhibition of HCG-stimulated accumulation of testosterone by rat testicular cells

Treatment	Testosterone accumulation (ng per culture)
None	0.04 ± 0.006
CPA (10 ⁻⁶ M)	0.03 ± 0.004
R1881	0.03 ± 0.002
CPA (10 ⁻⁶ M) + R1881	0.05 ± 0.005
HCG	3.74 ± 0.4
HCG + R1881	0.58 ± 0.07*
HCG + R1881 + CPA (10 ⁻⁶ M)	1.95 ± 0.2†
HCG + R1881 + CPA (10 ⁻⁵ M)	0.77 ± 0.1†

Testicular cells were cultured for 10 days as described in Fig. 1 legend. The cells were then reincubated for 2 additional days in the presence or absence of HCG (10 ng ml⁻¹), R1881 (5.6 × 10⁻⁶ M), cyproterone acetate or combinations thereof. Data points represent mean ± s.e., n = 4.

* Significantly different from cells treated with HCG ($P < 0.01$).

† Significantly different from cells treated with HCG plus R1881 ($P < 0.05$).

uncertain, androgens may exert acute and direct competitive inhibition at the level of the steroidogenic enzyme 17 β -hydroxysteroid dehydrogenase⁷. However, no such instantaneous end-product inhibition could be demonstrated during a 3-h incubation using an enriched Leydig cell suspension².

That cyproterone acetate is also endowed with progestational activity⁵ and that R1881 binds equally well to specific androgen and progestin binding sites^{5,8,9} in the rat prostate and rabbit uterus respectively suggest that the effects of R1881 and cyproterone acetate on testicular steroidogenesis may be mediated through progestin receptors. However, our findings that R5020 had no effect on testicular steroidogenesis argue strongly against the possibility that progestin receptors participate in the autoregulation of testicular androgen production. In contrast, the opposing effects of R1881 and cyproterone acetate suggest that autoregulation of testicular androgen production involves specific androgen receptors. This notion is further supported by our finding that cyproterone acetate can partially antagonize the R1881-induced inhibition of the HCG-stimulated accumulation of testosterone. Indeed, specific androgen receptors have been demonstrated in both Leydig^{10,11} and Sertoli^{12,13} cells. However, because both cell types are present in the testicular cell culture used here, one cannot exclude the possibility that the effect of

androgens on testicular steroidogenesis involves cell types other than the Leydig cell.

Our findings provide the first conclusive *in vitro* evidence for the existence of local autoregulation of testicular androgen production. Similar autoregulatory mechanisms have been reported in other steroidogenic tissues such as the adrenal^{14,15} and the corpus luteum¹⁶. In keeping with previous *in vivo* observations^{1,2}, testicular androgens seem to regulate their own production via an ultra-short loop negative feedback mechanism. This arrangement probably serves to limit androgen production in the face of inappropriate testicular stimulation and may therefore partake in desensitization¹⁷. Local autoregulation of androgen production may also account for the *in vitro* findings¹⁸ that reduction of testosterone accumulation through binding to plasma macromolecules increases testosterone secretion of perfused rabbit testes. It is tempting to speculate that autoregulation of testicular androgen production may be important in maintaining an optimal intratesticular androgen environment for spermatogenesis.

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Induction of ovulation *in vitro* by LH and catecholamines in hens is mediated by α -adrenergic receptors

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In the domestic hen, ovulation is preceded by an increase in plasma levels of luteinizing hormone (LH)^{1,2}, but the local events within the ovary which lead to follicular rupture are not clearly understood. The mechanism of ovulation is thought to involve enzymatic activity³, morphological change in the stigma⁴ and neuromuscular activity modulated by α -adrenergic receptors⁵. The administration of anti-adrenergic drugs *in vivo* inhibits ovulation^{6,7}, while the largest ovarian follicles contain high concentrations of noradrenaline and adrenaline, and low levels of dopamine⁸. We have now examined the possibility that these catecholamines, in addition to LH, are involved in the ovulatory process at the ovarian level. We found that noradrenaline and adrenaline induce ovulation independently of LH and that this action is mediated through α -adrenergic receptors at the follicle level. Dopamine does not seem to have any direct role in this process.

Table 2 Effects of treatment with R1881 or cyproterone acetate (CPA) on the choleragen and (Bu)₂-cyclic AMP-stimulated accumulation of testosterone by rat testicular cells

Treatment	Testosterone accumulation (ng per culture)
None	0.04 ± 0.01
Choleragen	2.4 ± 0.2
Choleragen + R1881 (10 ⁻⁵ M)	0.9 ± 0.1*
Choleragen + CPA (10 ⁻⁶ M)	5.6 ± 0.4*
Choleragen + CPA (10 ⁻⁵ M)	3.6 ± 0.1*
(Bu) ₂ -cyclic AMP	2.5 ± 0.3
(Bu) ₂ -cyclic AMP + R1881 (10 ⁻⁵ M)	0.1 ± 0.01†
(Bu) ₂ -cyclic AMP + CPA (3 × 10 ⁻⁶ M)	5.0 ± 0.3†
(Bu) ₂ -cyclic AMP + CPA (10 ⁻⁵ M)	3.1 ± 0.4†

Testicular cells were cultured for 10 days as described in Fig. 1 legend. The cells were then reincubated for 2 additional days with choleragen (10 ng ml⁻¹) or (Bu)₂-cyclic AMP (0.5 mg ml⁻¹) in the presence or absence of R1881 or cyproterone acetate. Data points represent mean ± s.e., n = 4.

* Significantly different from cells treated with choleragen ($P < 0.01$).

† Significantly different from cells treated with (Bu)₂-cyclic AMP ($P < 0.01$).

Table 1 Effects of incubation with various compounds on hen follicular ovulation

Treatment	No. of immature follicles incubated	No. of follicles ovulated
Control	7	0
LH (50 µg)	7	7
LH (50 µg) + phenoxybenzamine (10 ⁻⁴ M)	6	0
LH (50 µg) + propranolol (10 ⁻⁴ M)	7	6
Adrenaline (10 ⁻⁵ M)	6	6
Adrenaline (10 ⁻⁵ M) + phenoxybenzamine (10 ⁻⁴ M)	4	0
Adrenaline (10 ⁻⁵ M) + tolazoline (10 ⁻⁴ M)	6	0
Adrenaline (10 ⁻⁵ M) + propranolol (10 ⁻⁴ M)	8	6
Noradrenaline (10 ⁻⁵ M)	8	6
Noradrenaline (10 ⁻⁵ M) + tolazoline (10 ⁻⁴ M)	6	0
Dopamine (10 ⁻⁵ M)	6	0

Ovarian follicles were taken from hens laying regular sequences of more than three eggs and incubated *in vitro* to investigate the effect of LH and catecholamines on ovulation. Only the largest ovarian follicle in a follicular hierarchy was used and was estimated to be approximately 24 h from ovulation as indicated by the presence of the preceding ovum in the magnum. The follicles were removed from the donor hens immediately after the first egg of a sequence had been laid.

Single follicles were incubated at 41 °C for 12 h in 15 ml blood serum collected from immature male White Leghorn birds. Iproniazid phosphate (10⁻⁶ M) and tropolone (10⁻⁶ M) were added to the incubation medium to block monoamine oxidase⁹ and catechol-*O*-methyltransferase¹⁰ activities a few minutes before the addition of the follicles. LH (NIH-B10), noradrenaline and adrenaline (Sigma) were added 25 min after the addition of the adrenergic blockers. Details of the incubations and the observations recorded are given in Table 1.

Ovulation was induced by LH and this action was inhibited by the presence of phenoxybenzamine, an α -adrenergic receptor blocker, in the incubation medium, but not by propranolol, a β -adrenergic receptor blocker. Adrenaline and noradrenaline also induced ovulation and this action was blocked by phenoxybenzamine or tolazoline, another α -adrenergic receptor blocker, but not by propranolol. Dopamine at the dose used did not induce ovulation.

The present study shows that noradrenaline and adrenaline, acting via an α -adrenergic mechanism, contribute to the process leading to ovulation of a follicle. Furthermore, LH is unable to induce ovulation if ovarian α -adrenergic receptors are blocked. It is thus probable that LH interacts with noradrenaline or adrenaline at the target site to induce ovulation. Indirect evidence suggests that catecholamines are also involved in the induction of ovulation in humans, rabbits, sheep and cats¹¹⁻¹⁴. Our results provide direct evidence in this regard and our findings, although based on experiments with avian follicles, could have implications for other species.

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Potassium depletion decreases the number of ³H-ouabain binding sites and the active Na-K transport in skeletal muscle

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During potassium depletion in rats, the skeletal muscles lose potassium and gain sodium, whereas the Na-K contents of the liver¹, brain, cerebrospinal fluid^{2,3}, erythrocytes and heart⁴ remain virtually constant for several weeks. Since the selective loss of potassium ions from the muscles may result from inhibition of the active Na-K transport⁵, it is of interest to determine whether potassium depletion is associated with a reduced capacity for Na-K pumping. This study explores this possibility with measurements of ³H-ouabain binding and ⁴²K uptake in soleus and extensor digitorum longus muscles obtained from rats or mice during potassium deficiency induced either by K-free diet, a diuretic or a potassium-binding resin. Potassium depletion leads to a pronounced (up to 78%) and reversible decrease in the total number of ³H-ouabain binding sites and a reduced capacity for Na-K pump-mediated ⁴²K uptake. This decrease in the number of functional Na-K pumps may be of importance for the selective loss of potassium from skeletal muscle and its maintenance during potassium depletion. Furthermore, it favours the redistribution of digitalis glycosides from the periphery to the heart and provides an explanation for the increased digitalis toxicity seen in patients suffering from chronic potassium depletion.

All experiments were performed using fed female Wistar rats (60-240 g) or NMRI mice (25-40 g). Potassium deficiency was induced by maintaining the animals on Altromin pellets containing 0.75 mmol K per kg and distilled water. In some experiments, a more rapid depletion was achieved by combining this fodder with the oral administration (via a polyethylene tube) of bumetamide (250 mg per kg body weight per day) or a potassium-binding resin (Resonium A 1,000 mg per kg per day). K repletion was induced by the oral administration of a 4M KCl solution three times daily (36 mmol per kg per day).

Soleus and extensor digitorum longus muscles were dissected out as described previously^{6,7} and equilibrated in Krebs-Ringer bicarbonate buffer under continuous gassing with a mixture of 95% O₂ and 5% CO₂. In the experiments with mice and young rats (60-70 g), the whole muscle was incubated. To ensure adequate oxygenation in the experiments with muscles from larger rats, the lateral segments of the soleus muscles were carefully dissected out and incubated as described elsewhere⁸. It has been shown that such muscle segments maintain almost normal ATP contents for 2 h *in vitro*⁸. Control experiments with 65 and 100 g rats showed that in muscle segments, the number of ³H-ouabain binding sites per g wet weight was the same as in the intact muscles (difference 2.4%, eight controls and eight trial observations).

The total number of ³H-ouabain binding sites was determined by measuring the specific (displaceable) binding of ³H-ouabain after 2 h of incubation in potassium-free Krebs-Ringer bicarbonate buffer containing 5 mM D-glucose⁹⁻¹¹. The Na-K contents of tissues, packed erythrocytes and plasma were determined by flame-photometry of trichloroacetic acid extracts^{6,12}, and ⁴²K uptake mediated by the Na-K pump was measured as described elsewhere¹².

In both rats and mice, maintenance on a potassium-deficient diet induced a rapid reduction in the potassium content of plasma and skeletal muscle (Fig. 1). In contrast, the brain lost no potassium, and in the heart, only modest losses were observed after 3-4 weeks. The erythrocytes and the liver showed virtually

Table 1 Effect of potassium depletion and repletion on Na-K pump-mediated ^{42}K uptake and the number of ^3H -ouabain binding sites in rat soleus muscle

	^{42}K uptake ($\mu\text{mol per g wet wt per min}$)		^3H -ouabain binding sites (pmol per g wet wt)		^{42}K uptake (ions per ouabain binding site per min)
Controls	0.377 ± 0.034 (10)		254 ± 17 (6)		$1,484 \pm 249$
K-depleted for 3 weeks	0.154 ± 0.011 (10)	$P < 0.001$	72 ± 4 (8)	$P < 0.001$	$2,139 \pm 288$
K-depleted for 3 weeks, repleted for 1 day	0.197 ± 0.009 (4)	$P < 0.05$	107 ± 5 (16)	$P < 0.001$	$1,841 \pm 179$

Rats weighing 130–170 g were maintained on potassium-deficient diet for 3 weeks. One group was given KCl (36 mmol per kg body weight) and the remaining kept on the potassium-deficient diet. The lateral segments of the soleus muscles were incubated in Krebs–Ringer bicarbonate buffer containing 5 mM D-glucose for 75 min. After this equilibration period, during which the muscles from the K-deficient animals regained K to 96% of the control level, the tissues were transferred into tubes containing the same buffer without or with ouabain (10^{-3} M). After an equilibration period of 15 min, a trace amount ($0.2 \mu\text{Ci ml}^{-1}$) of ^{42}K was added and the amount of ^{42}K taken up during the following 20 min of incubation determined as described previously^{10,12}. The ouabain-suppressible component of ^{42}K uptake was calculated as the difference between the uptake measured in the absence and the presence of ouabain. The results are given as mean values $\pm$ s.e. with the number of observations in parentheses.

no decrease in potassium content after 4 weeks on potassium-deficient fodder. After 3–4 weeks of depletion, potassium levels in the gastrocnemius muscle and plasma appear to reach a plateau which is maintained for up to 8 weeks. In the soleus and extensor digitorum longus muscles, the relative falls in potassium content were of similar magnitude to those in the gastrocnemius. Concomitantly, the muscles gained an equivalent amount of sodium.

Figure 2 illustrates the effect of potassium depletion on the total number of ^3H -ouabain binding sites in rat soleus. Within 3 days there is a significant reduction, lasting for up to 8 weeks. In young rats (60–70 g) the combination of potassium-deficient diet and Resonium A for 1 week reduced the total number of

^3H -ouabain binding sites in whole intact soleus and extensor digitorum longus muscles by 38.5 and 33.7% respectively ($P < 0.001$; four groups of four observations). In mice, which were maintained on potassium-deficient diet and potassium-binding resin or bumetamide for 1 week, the total number of ^3H -ouabain binding sites in soleus and in extensor digitorum longus muscles were decreased by 22.5 and 41.2% respectively ($P < 0.05$ and < 0.001 ; three groups of five observations).

The effect of potassium depletion (3 weeks) on the number of ^3H -ouabain binding sites was also assessed in experiments with higher concentrations of ouabain or longer incubations. With 1, 2 or 5×10^{-6} M ^3H -ouabain, the decrease in the number of binding sites was 65.9, 78.3 and 63.5% respectively (six groups of six observations). With incubation periods of 120 and 360 min, the decrease was 65.5 and 52.8% respectively (four groups of four observations).

After 3 weeks of potassium depletion, the readministration of potassium increased the number of ouabain binding sites at a somewhat slower rate than the recovery of plasma and muscle potassium. As shown in Fig. 3, the control level of binding sites was not reached even after 4 days of potassium administration.

Potassium depletion also reduced the ouabain-suppressible (Na-K pump mediated¹²) ^{42}K uptake, but, as shown in Table 1, the relative decrease is not as pronounced as the drop in the number of ouabain binding sites. Following 1 day of potassium repletion, the rise in the number of ouabain binding sites is associated with a smaller increase in the ouabain-suppressible ^{42}K uptake. The increased number of ^{42}K ions pumped per ^3H -ouabain binding site in the muscles obtained from potassium-depleted or potassium-depleted/repleted animals is possibly the result of the intracellular Na levels remaining somewhat elevated, even after the pre-equilibration period *in vitro*.

This study confirms the finding of a selective loss of potassium from skeletal muscles during potassium depletion. The major new finding is that this is associated with a pronounced drop in the total number of ^3H -ouabain binding sites. Since almost the same relative decrease was found using longer incubation or a five-fold range of ouabain concentrations, it is unlikely to reflect a change in the affinity of the ($\text{Na}^+ + \text{K}^+$)-activated ATPase for ouabain. This, together with the observation that the ouabain-suppressible ^{42}K uptake is diminished, indicates that skeletal muscles respond to potassium depletion with a decrease in the number of Na-K pumps as well as a reduced capacity for performing active Na-K transport. This may be of significance for the release of potassium from the muscles and for the maintenance of their low potassium contents in conditions where vital organs demand potassium for their continued function⁵. Furthermore, at low extracellular potassium levels, the muscle cells may also hyperpolarize and lose excitability unless their intracellular K concentration is decreased.

At variance with our results, potassium depletion has been shown to increase ($\text{Na}^+ + \text{K}^+$)-activated ATPase as well as the number of ouabain binding sites in erythrocytes^{13,4}, and a similar

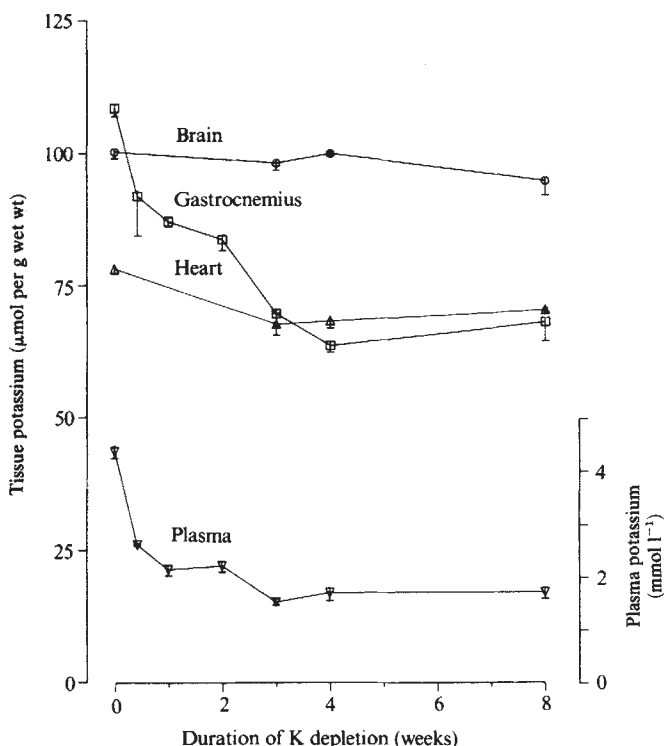


Fig. 1 Time course of the changes in potassium contents of tissues and plasma during potassium-depletion. Rats (110–240 g) were maintained on potassium-deficient diet (Altromin) for the periods indicated. Blood samples were withdrawn from the carotid artery under light ether anaesthesia, collected into glass tubes wet with a heparin solution (30 IU ml^{-1}) and centrifuged. Tissue samples were taken immediately after decapitation, homogenized in 5% trichloroacetic acid and centrifuged. The potassium and sodium contents of the plasma and the trichloroacetic acid extracts were determined by flame photometry using a Radiometer FLM 3 flame photometer with lithium as internal standard. Each point represents the mean of 2–14 observations with bars denoting s.e., where this exceeds the size of symbols.

compensatory phenomenon has been observed in cell cultures^{14,15}. An analogous response might account for the persistence of the normal potassium content in erythrocytes observed here. The paradoxical response of the muscles suggest that in this tissue, the synthesis of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ is regulated by a humoral or nervous factor. The observation that in potassium-depleted rats, denervation of soleus muscles leads to a selective recovery of their intrafibre potassium within 6 hours⁵, point to the existence of a central control of active Na-K transport in skeletal muscle.

It is well known that chronic potassium depletion is associated with increased digitalis toxicity^{16,17}. The present results indicate that potassium depletion leads to a considerable decrease in the binding capacity of skeletal muscles for digitalis glycosides. Since this tissue represents the largest single pool for the peripheral binding of digitalis, potassium depletion is likely to

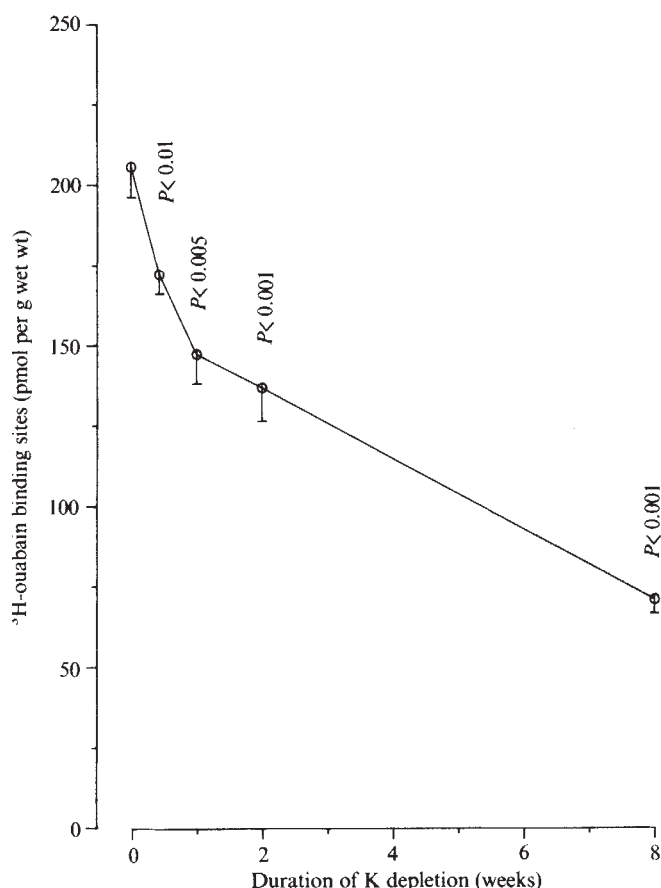


Fig. 2 Time course of the changes in the total number of ^3H -ouabain binding sites in soleus muscles from rats maintained on potassium-deficient diet. Rats (210–240 g) were killed by decapitation and the lateral segments of their soleus muscles incubated for 120 min in potassium free Krebs–Ringer bicarbonate buffer containing 1.27 mM Ca, 5 mM D-glucose and 2×10^{-6} M ^3H -ouabain ($0.6 \mu\text{Ci ml}^{-1}$) at 30°C under continuous gassing with a mixture of 95% O_2 and 5% CO_2 . Muscles were washed four times for 30 min at 0°C to remove ^3H activity from the extracellular space, blotted, weighed and homogenized in 2 ml of 5% trichloroacetic acid. After centrifugation, 1 ml of the clear supernatant was taken for liquid scintillation counting of the ^3H activity. On the basis of the specific activity of the incubation medium, the amount of ^3H activity retained following the cold wash was expressed as pmol per g wet wt. A minor correction for nonspecific retention of ^3H activity was based on measurements performed by incubating the contralateral muscle segments at 10^{-3} M ^3H -ouabain (for details see refs 9–11). Each point represents the mean of 4–12 observations with bars denoting s.e. The significance of the difference between the results obtained using age-matched controls maintained on normal diet and those of the K-depleted rats is given above each point.

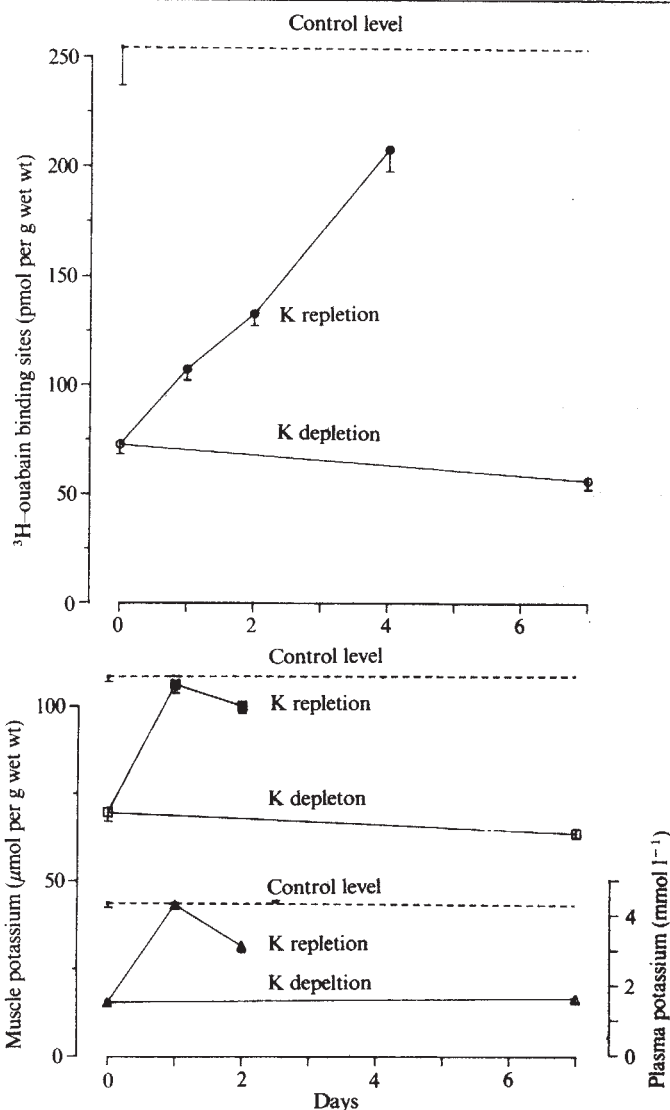


Fig. 3 Time course of changes in the total number of ^3H -ouabain binding sites and the potassium contents of plasma and gastrocnemius during potassium repletion. Rats (110–170 g) were maintained on potassium-deficient diet for 3 weeks. One group of animals were kept on this diet for another week, others were given KCl (36 mmol per kg per day) for either 1, 2 or 4 days. Potassium contents and ^3H -ouabain binding were determined as described in Figs 1 and 2. Each point represents the mean of 2–16 observations with bars denoting s.e. Dashed lines indicate control levels measured in rats on normal diet.

favour a redistribution of the drug leading to higher plasma concentrations and preferential binding to the heart.

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A family of T-cell alloantigens linked to Igh-1

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Tsu^d (ref. 1) is an alloantigen on mouse suppressor T cells, coded for by a gene downstream from the immunoglobulin locus on chromosome 12². We now report a second T-cell alloantigen, **Tind^d**, which is closely linked to **Tsu^d**. Both antigens may be products of a family of genes coding for conserved, non-antigen binding site regions of T-cell receptors. **Tind^d** is defined by a monoclonal BALB/c (Igh-1^a) anti-C.AL-20 (Igh-1^d) antibody and is expressed preferentially on Lyt 1⁺2⁺3⁺ peripheral lymphoid cells, whereas **Tsu^d** is expressed on Lyt 1⁺2⁺3⁺ suppressor cells. The interpretation that these determinants represent antigen binding molecules is in part based on the antibody-mediated induction of cell function. *In vivo* treatment of C.AL-20 animals with anti-Tsu^d antiserum at day 4 suppresses the primary immune response to sheep red blood cells (SRBCs)³⁻⁵. Pretreatment with limiting dilutions of anti-Tind^d antibody induces cyclic waves of either suppression or enhancement of the plaque-forming cell response. The genes coding for these determinants have now been mapped in inbred congenic mice having recombination events in or near the immunoglobulin locus. Monoclonal anti-Tind^d antibodies precipitate a group of polypeptides of molecular weights (MWs) 62,000, 45,000 and 17,000 from ³⁵S-methionine-labelled spleen cells activated with concanavalin A (Con A) whereas the polypeptides associated with the **Tsu^d** specificity have MWs 69,000, 45,000 and 25,000. The differences in molecular weights and contrast in the Lyt phenotype of cells expressing these antigens suggest they are separate molecules and may imply discrete isotypes of T-cell receptor.

We previously described the production of an alloantiserum which may recognize the constant region of a T-cell receptor for antigen¹⁻⁵. Antibody directed against the Igh-1-linked alloantigen blocks the binding of Ts₂ cells from hyperimmune suppressed mice⁶ to the Ars IdX⁷ and acts as a polyclonal activator of suppressor T cells for T-dependent antigens⁴. If antibody binds to a constant region of the receptor on cells for all antigens, then cross-linking of the cell surface might trigger the cells⁸ in the observed antigen-independent manner⁴. The genetic restrictions imposed on the antibody-induced⁵ suppressor cells mirror those reported previously for regulatory cells for major cross-reactive idiotypes⁹. Antisera having these properties were used to precipitate a ³⁵S-methionine-labelled complex of antigens from extracts of spleen tissue cultures from allotype congenic pairs of mice (Fig. 1). The major species precipitated is a 69,000-MW polypeptide, although antigens of MWs 45,000 and 25,000 co-precipitated in variable c.p.m. ratios in different cell preparations. The structural relationship between these antigens is unclear, although the appearance of the 69,000-MW molecule critically depends on the use of proteolytic inhibitors throughout the preparation preceding electrophoresis. Figure 1 shows that these antigens are C.AL-20 specific and are not present on the BALB/c cell, as predicted by the strain pattern¹ of induction of suppressor T cells.

We have now produced a monoclonal antibody using a modification of this immunization protocol. The γ 1 κ -secreting line, 9IIIA2, produces an antibody (anti-Tind^d) which recognized an antigen(s) on C.AL-20 cells that is similar to but antigenically distinct from **Tsu^d**. The antigen (**Tind^d**) comprises polypeptides of MWs 62,000, 45,000 and 17,000 and is detected in a strain-specific pattern (Fig. 2). BALB/c and C.B.AL-1

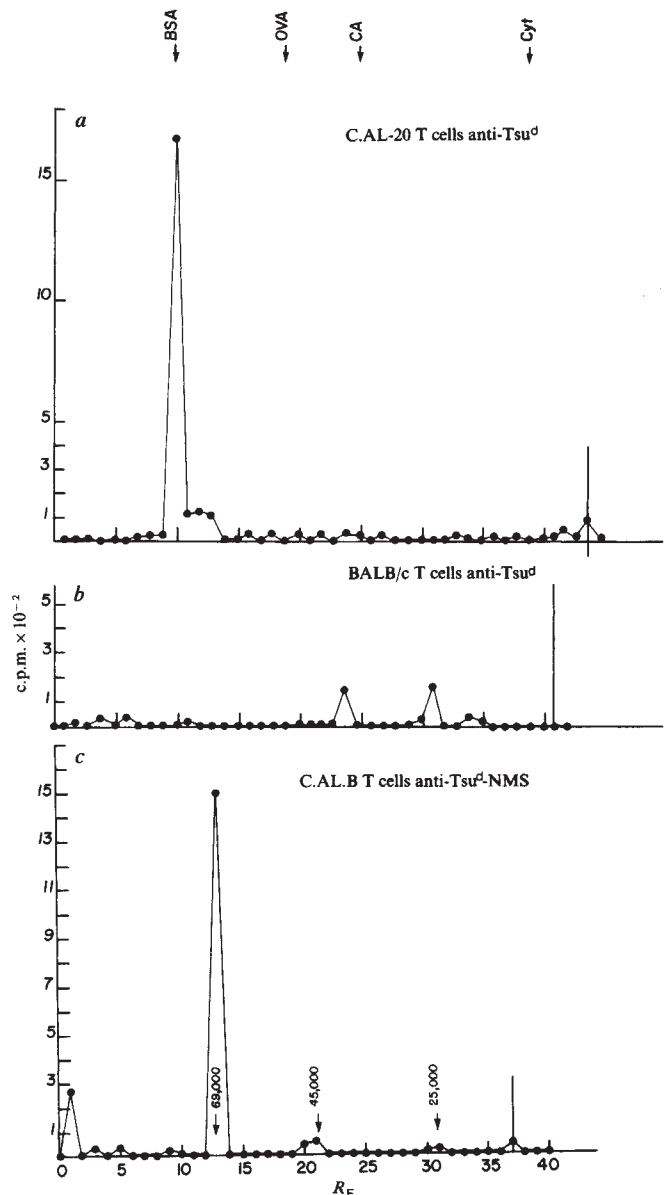


Fig. 1 SDS-polyacrylamide gel electrophoresis of ³⁵S-methionine-labelled antigens synthesized by splenic cells stimulated with Con A for 48 h from: a, C.AL-20 (Igh-1^d); b, BALB/c (Igh-1^a); c, C.AL.B (Igh-1^d, H-2^b)²⁰ animals. Cells (2×10^7 ml⁻¹) were metabolically labelled by incubating washed cells in methionine-free RPMI for 4 h with 100 μ Ci of ³⁵S-methionine and washing three times with phosphate-buffered saline (PBS) before extracting. Cell extracts were obtained by lysing washed cells three times in 1 ml of buffer containing 0.01 M NaPO₄, 0.1 M NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS and 0.0025 M phenylmethylsulphonyl fluoride. Supernatants from a 35,000g centrifugation (90 min) of cell extracts were incubated with 5 μ l of anti-Tsu^d antiserum chosen from early bleeds of selected sera known to contain the biological activity for the **Tsu^d** gene product¹⁻⁴ or with 5 μ l of normal mouse serum. Extracts were incubated with glutaraldehyde-fixed *Staphylococcus aureus* organisms (Immunsorb) precoated with sheep anti-mouse IgG1-antiserum. Labelled antigen was eluted from washed adsorbent in buffer containing 5% SDS, 0.25 M Tris pH 6.8, and 2.5% 2-mercaptoethanol and electrophoresed on 12% tube acrylamide gels²¹ cross-linked with DATD²² in Tris-glycine buffer for 2.5 h at a constant current of 3 mA per gel at 25 $^{\circ}$ C. Sliced gels were incubated with 5% Protosol in Econofluor scintillation fluid from 12 h before counting at 25 $^{\circ}$ C.

animals, which were previously shown to lack the **Tsu^d** gene, also lack this alloantigen. The C.B.AL-1 mouse has documented recombination events on chromosome 12 which limit the position of the gene(s) coding for the new specificity to a region of chromosome 12 between *V_HDex* and a recombination event between **Tsu^d** and prealbumin¹⁰ (Table 1b). This region encompasses the proposed IgT region¹. The close proximity of **Tsu^d** and **Tind^d** suggests that a family of T-cell-specific polypeptides may be encoded here. Further investigation of

hyperimmune anti-Tsu^d alloantisera identifies the second Tind^d specificity as a 'contaminant' band in precipitation studies and also shows a larger band in C.AL-20 but not BALB/c cells, of unknown function. Monoclonal antibody or alloantisera coupled to Sepharose 4B was used to preabsorb cell extract before precipitation with anti-Tind^d or anti-Tsu^d antibody. Coupling of anti-Tind^d antibody to Sepharose 4B and preclearing cell extracts before addition of anti-Tsu^d serum removes the 62,000- and 17,000-MW peaks from the ³⁵S-methionine-precipitated material but does not preclear the 69,000- and 25,000-MW polypeptides. Therefore, the 62,000-, 45,000- and 17,000-MW polypeptides precipitated with anti-Tsu^d are those molecules recognized by anti-Tind^d. The 69,000-, 45,000- and 25,000-MW molecules do not appear to cross-react serologically with the 62,000-, 45,000- and 17,000-MW molecules.

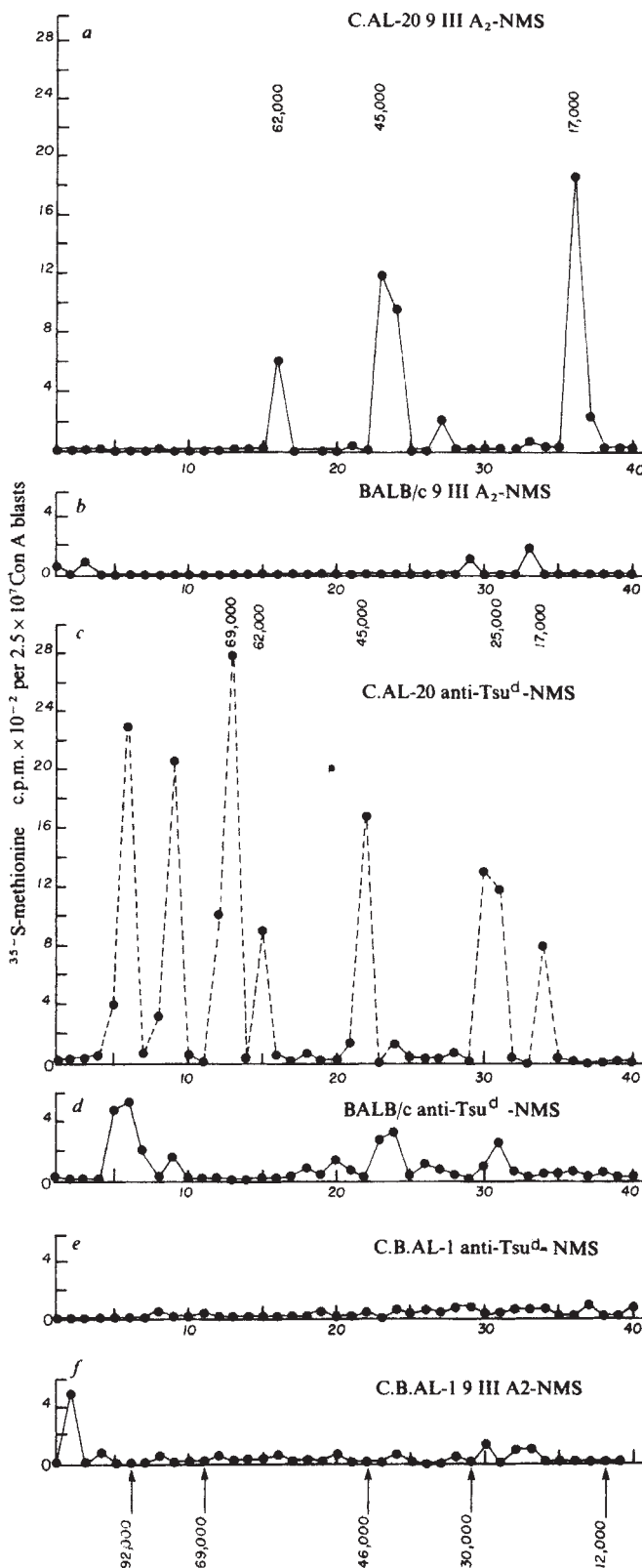
The T-cell determinants recognized by anti-Tsu^d or anti-Tind^d antibody are not expressed on serum immunoglobulins. Antisera directed against immunoglobulin isotypes (summarized in Fig. 2) do not preclear or co-precipitate these antigens. In addition, ammonium sulphate-precipitated ascites protein from clone 9IIIA2 (anti-Tind^d) was labelled with ¹²⁵I and incubated with plates coated with either BALB/c or C.AL-20 normal mouse immunoglobulin. Anti-Tind^d antibody fails to bind serum immunoglobulins.

The biological properties of the cell(s) displaying Tind^d have not been defined. Visual fluorescence staining experiments suggest that 3–5% of mature peripheral T cells eluted from nylon wool are positive. Negative selection with monoclonal anti-Lyt-1.2 or anti-Lyt-2.2 antibodies and complement followed by visual surface fluorescence suggests preferential expression of this determinant on an Lyt-1.2 high-density cell in contrast to the preferential expression of Tsu^d on Lyt-2.2 cells^{2,3}. Staining of thymocytes, bone marrow or splenic B cells by monoclonal anti-Tind^d antibody cannot be detected visually.

In vivo pretreatment of mice with anti-Tind^d antisera before injection of SRBCs either enhances (induction of help) or reduces (induction of suppression) the response. The cyclic induction of an altered response depends on when the antiserum is administered. The results are reminiscent of the hypothetical waves of suppressor and helper cells postulated to interact in regulating the idiotype response of mice^{11,12} and may represent either sequential steps or biological alternatives in a regulatory network¹³. An Lyt-1⁺ inducer T cell which initiates Lyt-2⁺, 3⁺-mediated suppression was hypothesized¹⁴, although no relationship between Tind^d and that cell has been tested experimentally.

Fig. 2 Monoclonal antibody against Tind^d from clone 9IIIA2 precipitates a complex of antigens with a molecular weight distinct from that of Tsu^d. This monoclonal cell line was obtained by fusion of spleen cells from mice immunized with a protocol similar to that reported to raise antibody to Tsu^d (refs 1–4). BALB/c AnN mice were immunized twice intravenously (i.v.) with C.AL-20 cells activated with Con A and fused 3 days after the last injection. Spleen cells from immune donors were fused with BALB/c myeloma P.3U1 (ref. 23), a non-secreting variant of a BALB/c myeloma, with 30% polyethylene glycol. Fused cells were grown in HAT (hypoxanthine, aminopterin and thymidine), Dulbecco's high glucose medium with 10% horse serum in 96-well microtitre plates²⁴. The fusion frequency was 30% and 9IIIA2 cells subcloned on thymocyte feeder layers had a 50% cloning efficiency. Subclones develop in 10–12 days as visible clones. The hybridoma line has been subcloned sequentially three times and grown *in vitro* for 6 months as a secreting line. Subcloned lines are maintained in 10% horse serum in Dulbecco's H-21 medium with essential amino acids, non-essential amino acids, sodium pyruvate, glutamine, penicillin/streptomycin. *c*, *d* And *e* show later bleeds of the same alloantisera shown in Fig. 1 (additional strain specific molecules are detectable). The antigens precipitated by clone 9IIIA2 (frame *a*, *b*, *f*) correspond to the 62,000-, 45,000- and 17,000-MW species detectable in the late bleeds of the alloantisera. The following control antisera were ineffective in blocking or preabsorbing the molecules precipitated by anti-Tsu^d antibody: affinity-purified class-specific anti-isotype reagents²⁵ (rabbit anti-mouse IgG2a, anti-HOPC-1, 25 µg binding capacity (b.c.); sheep anti-mouse IgG3, anti-J606, 250 µg b.c.; goat anti-mouse IgM, anti-ABPC-22, 720 µg b.c.; rabbit anti-IgM, anti-ABPC-22, 10 µg b.c.; rabbit anti-mouse IgD, anti-MOPC 1017, 5 µl serum or 50 µg Fab fragments of affinity-purified antibody); monoclonal anti-Thy-1.2, 10⁸ lytic units; monoclonal anti-Lyt-1.2, 10⁸ lytic units; goat anti-Rauscher GP70 serum, 5 µl.

Cyclic waves of enhancement or suppression are induced when 5 ng of antibody is administered at different times over 10 days. The time of appearance of the crest of these waves varies between experiments but the magnitude of the crest is constant (Fig. 3). In fact, one observes either the pattern in Fig. 3*a* or that in Fig. 3*b*; they are mirror images. In contrast, when the time (day 4) is held constant and the antibody concentration varied, 5 µl of tissue culture supernatant (~5 ng of immunoglobulin) showed a statistically significant strain-specific enhancement for the C.AL-20 animal (Table 1). The normalized grand geometric



mean $\pm$ s.e. of 15 mice in five separate experiments was 52,987 plaque-forming cells (PFC) $\pm$ 1.23 for control values and 108,710 $\pm$ 1.47 for those treated with 5 ng of antibody ($P = 0.001$). The kinetics of enhancement change rapidly (Fig. 3); the need to assay during a critical few hours in the immune response may explain why the changes range from two- to fourfold in different experiments. Table 1b shows the recombination event in the C.B.AL-1 animal which limits the gene coding for this biological activity to the region of chromosome 12 mapped for *Tsu*^d (ref. 1). As only low doses of antibody enhance the

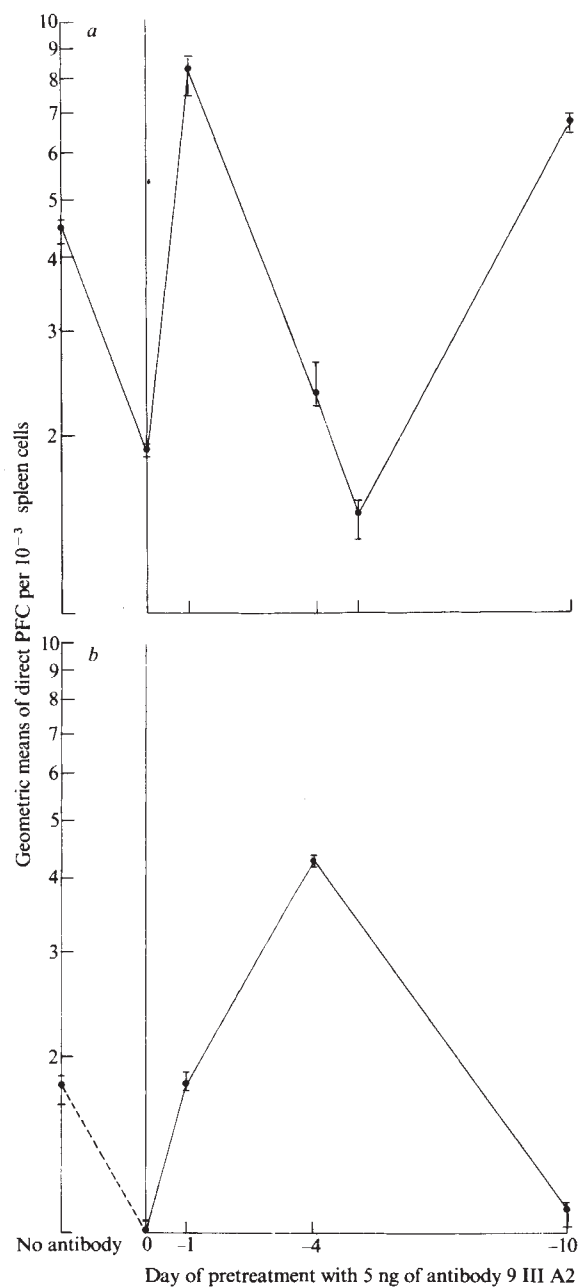


Fig. 3 Monoclonal anti-Tind^d antibody injected into the tail vein of recipient mice induces a cyclic pattern of either suppression or enhancement of the primary PFC response against SRBCs. The data points show the effect of injecting antibody (~5 ng) from tissue culture supernatants of clone 9IIIA2 into each of three C.AL-20 adult recipients at days 0, 1, 4, 5, or 10 and challenging those recipients with 10⁸ SRBCs in PBS on day 0. In contrast to results reported earlier for antibody to *Tsu*^d (refs 2, 3) where injection at day 4 of low doses of antibody was effective and other injections in this time spectrum showed no alteration in the response, injection of anti-Tind^d seems to have a reproducible dual role in the response. Data points represent the geometric mean $\pm$ s.e., $n = 3$ mice. Three similar experiments yielded the same pattern with variations in the lag period before enhancement from 1 to 4 days. *a* and *b* represent two separate experiments and show the variation in kinetics of this regulation using the same reagents. These cyclic waves are strain specific for C.AL-20 but not for BALB/c.

Table 1 Strain-specific enhancement of the PFC response to SRBCs limits the gene coding for the Tind^d molecule to the IgT region of chromosome 12

<i>a</i>	9IIIA2* (ng)	Strain		
		C.AL-20	BALB/c	C.B.AL-1
	(control)	21,000 $\pm$ 1.02	74,903 $\pm$ 1.79	56,475 $\pm$ 1.34
	0.1	35,327 $\pm$ 1.15	88,545 $\pm$ 1.15	58,000 $\pm$ 1.10
	1.0	42,085 $\pm$ 1.03	ND	58,240 $\pm$ 1.21
	2.0	44,348 $\pm$ 1.17	69,654 $\pm$ 1.10	58,780 $\pm$ 1.22
	5.0 $P = <0.001$	53,665 $\pm$ 1.28†	73,010 $\pm$ 1.05	58,981 $\pm$ 1.10
	50.0	25,258 $\pm$ 1.08	89,000 $\pm$ 1.03	55,300 $\pm$ 1.12
	200.0	20,080 $\pm$ 1.03	ND	ND
<i>b</i>				
		Genotype of strains used		
C.AL-20		<i>Vh</i> ^d <i>Igh</i> - 1 ^d <i>Tsu</i> ^{d+} <i>Pre</i> ⁰		
BALB/c		<i>Vh</i> ^d <i>Igh</i> - 1 ^a <i>Tsu</i> ^{d-} <i>Pre</i> ⁰		
C.B.AL-1		<i>Vh</i> _x ^d <i>Igh</i> - 1 ^b <i>Tsu</i> _x ^{d-} <i>Pre</i> ⁰		

* Monoclonal antibody directed against Tind^d (clone 9IIIA2) was injected i.v. in recipient mice differing genetically in the IGH-1 linked genes (see *b*). A range of dilution of tissue culture supernatant containing antibody was injected on day 4 and the primary day-3 PFC response of mice challenged i.v. with 10⁸ SRBCs on day 0 was measured. Two of the C.AL-20 (*Igh*-1^d) mice were enhanced for the geometric mean response (5 ng TCS) in comparison with control mice not treated with antibody (0 ng). These data are from one of five experiments showing a similar pattern of stimulation. PFC values were obtained by the Cunningham plaque chamber assay²⁶ as previously reported^{2,3}.

† The normalized grand geometric mean $\pm$ standard error of 15 mice in five separate experiments was 52,987 $\pm$ 1.23 for control value but 108,710 PFC $\pm$ 1.47 for those treated with 5 ng of antibody ($n = 15$, $P = <0.001$ in double-tailed Student's *t*-test).

response, the antibody probably activates or stimulates a cell, possibly cross-linking that cell membrane through recognition of conserved structures on the receptor or 'constant regions'. Therefore, both *Tsu*^d alloantisera and Tind^d monoclonal alloantibody appear to act as activators for regulatory cells for the immune response. The kinetics of activation by these two reagents are very different^{2,3}. We cannot exclude the possibility that both antigens are on mutually overlapping cell populations, but the visual typing suggests they are on separate cells with related or tandem functions.

The molecular weights of *Tsu*^d and Tind^d are similar to those described for SRBC suppressor factor secreted from an Lyt-2, 3⁺ cell line maintained with T-cell growth factor (MW 69,000)¹⁵ and the product of a hybrid T-cell line secreting a molecule (MW 62,000) specific for the Ars hapten¹⁶.

These data imply that two similar, but serologically distinct, T-cell-specific antigens are encoded by closely linked genes on chromosome 12. We have not proved that these antigens are T-cell receptors for antigen nor has Tind^d been mapped by recombination. It is possible that the markers *Tsu*^d and Tind^d are unique differentiation antigens involved in the expression of cell-surface antigens, facilitating expression of antigen-binding molecules. Lyt-2 differentiation antigens are co-expressed with antigen receptors on cytotoxic T cells^{17,18}. However, we favour the hypothesis that these antigens represent constant regions on T-cell receptors for antigen¹⁹ with some analogy to the immunoglobulin heavy chains. The map positions of these genes suggest they may have arisen from the same primordial domain as the immunoglobulin heavy-chain genes and were evolutionarily modified to function in cellular recognition.

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Family studies define a new histocompatibility locus, *SB*, between *HLA-DR* and *GLO*

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Gene products of the human *HLA* region are the principal histocompatibility barrier to human allogeneic tissue transplantation¹. In addition, *HLA* genes regulate immune responses to environmental antigens² and affect susceptibility to various diseases³. Although the *HLA* region has been studied intensively, only four histocompatibility loci (*HLA-A*, *-B*, *-C* and *-DR*) have been identified within this 'supergene', but the existence of additional loci has been suggested by immunochemical⁴⁻⁹ and genetic recombination¹⁰⁻¹⁵ studies. Recently, a new segregant series of five histocompatibility antigens, named 'secondary B cell' (SB) antigens, was defined¹⁶⁻¹⁸. We now report studies of 21 families which indicate that the gene encoding the SB antigens is closely linked to *HLA-DR*, but is distinguished from it by recombination in two families and can be mapped between *HLA-DR* and *GLO* in additional recombinant families.

The SB antigens are identified by a primed lymphocyte typing (PLT) system which has been optimized to ensure reproducible typing¹⁸. The 'reagents' used to define the five SB antigens are sets of cryopreserved primed lymphocytes from 10 different donor combinations, and include two reagents to define each of the five SB antigens. The same reagents have been used to SB type over 500 donors; the agreement between results for the two reagents defining each SB antigen is high ($r > 0.86$). A donor's cells are SB typed by using them to restimulate these 10 populations of primed cells; the assignment of SB specificities is then made by objective statistical analysis¹⁸ (although the appropriate scoring is generally apparent visually).

Population studies suggest that the five SB antigens are a simple mendelian segregant series of antigens in Hardy-Weinberg equilibrium¹⁸. The SB phenotype of donors was largely independent of their *HLA-A*, *-B* and *-DR* phenotypes¹⁸. Furthermore, family studies indicate that there is no statistically significant association between SB markers and the *HLA-A*, *-B* and *-DR* markers on 84 haplotypes. These data indicated that SB antigens might be products of a new locus, and this proposition has now been confirmed by two independent lines of inquiry: the family studies reported here and analysis of mutant cell lines¹⁹.

Table 1 *SB/DR* recombination in family Fa

PLT reagents	Restimulating cells and their <i>HLA</i> haplotypes						
	A <i>cd</i>	B <i>ab</i>	J <i>ac</i>	F <i>bc</i>	G <i>bd</i>	I <i>b/ad</i>	D <i>ad</i>
SB1A	86	26	80	57	7	23	51
SB1B	79	56	87	72	3	55	57
	+	+	+	+	-	+	+
SB2A	4	3	5	5	6	4	7
SB2B	14	3	7	9	10	7	7
	-	-	-	-	-	-	-
SB3A	3	8	4	6	2	7	4
SB3B	8	6	13	12	2	6	6
	-	-	-	-	-	-	-
SB4A	22	2	9	10	4	5	5
SB4B	10	3	9	6	4	6	5
	-	-	-	-	-	-	-
SB5A	22	16	22	26	5	11	12
SB5B	8	5	4	20	8	3	2
	-	-	-	-	-	-	-
SB assignment	1	1	1	1	b1	1	1

HLA haplotypes: *a*, *Aw24 B14 DR3 Dw3 SB1 Glo*2*; *b*, *A11 B35 DRw6 Dw6 SBblank Glo*1*; *c*, *A23 B41 DRw5 Dw5 SB1 Glo*2*; *d*, *A30 Bw16 DRw6 Dw6 SBblank Glo*1*; *b/a*, *A11 B35 DRw6 Dw6 SB1 Glo*2*. The 10 SB-specific PLT reagents were restimulated with peripheral blood lymphocytes from donors as previously described¹⁶⁻¹⁸. Numeric data are given as % relative response compared with a control positive stimulating cell; absolute c.p.m. for control responses (100% reference) ranged between 10,006 and 99,773. The determination of whether an antigen is present (+) or absent (-) was made by cluster analysis scoring of both reagents defining a specificity¹⁸. Family Fa has been typed using reagents of the 8th International Workshop and typed twice for *GLO*^{10,11}.

Studies of 21 families indicate that the SB antigens are encoded by a gene which is linked to *HLA* (mostly closely to *HLA-DR*) but is separable by recombination from all known markers in this region. In the generation of the 77 offspring tested, there had been 80 meioses which could be evaluated for the segregation of *SB*, including 8 previously known to involve recombination in this region: 4 between *HLA* and *GLO* and 4 within the known *HLA* region. In 70 of the other 72 informative meioses, *SB* segregated with all *HLA* and *GLO* markers; data on the remaining two are best explained by recombination between the known *HLA* genes and a new gene tightly linked to *HLA*.

Previous family studies^{16,17} have not separated the gene which encodes the SB antigens from *HLA-DR*; now we describe two families showing recombination between *SB* and *DR*. The first family (Fa) is well characterized and previously described by Mawas *et al.*^{10,11}. The recombination in this family divided the '*HLA-D* region' into two subregions: a telomeric region (*D-α*) which included *HLA-DR*, and a centromeric region (*D-β*) which included another gene(s) encoding antigens that induce allogeneic cellular proliferative responses. Members of this family were typed for SB antigens (Table 1) to determine whether the recombination would help to locate *SB*. The data from the parents and the non-recombinant siblings indicate that the *SB1* allele is present on both the *a* and *c* haplotypes, whereas the *b* and *d* haplotypes lack defined SB alleles; this is inferred from the fact that all family members type as SB1 only, except for the *ac* sibling (G) who has no defined SB antigens. The data on the recombinant sibling I map *SB1* centromeric to *HLA-DR*. The SB1 typing of the recombinant donor I resembles that of her *GLO*-identical (*ad*) sibling (D) much more than her serotypically identical (*bd*) sibling (G). Thus, sibling I inherited *SB1* together with *GLO*2* from the *a* haplotype but inherited the *DR6*, *B35* and *A11* alleles from the *b* haplotype.

The second family (Kav) shows recombination between *DR* and *SB* (Table 2). The mother's cells (KAV1) were positive for both SB2 and SB3. However, the three children with the maternal *c* haplotype (*A1*, *B8*, *DR3*) as well as the child

Table 2 SB/DR recombination in family Kav

PLT reagents	Restimulating cells and their HLA haplotypes											
	KAV	KAV	KAV	KAV	KAV	KAV	KAV	KAV	KAV	KAV	KAV	KAV
	1	2	3	4	5	6	1	2	3	4		
	cd	ab	ad/c	ac	bc	bc	cd	ab	ad/c	ac		
	Expt 1						Expt 2					
SB1A	19	35	10	8	8	10	3	8	2	2		
SB1B	20	17	4	2	3	5	8	6	1	2		
	—	—	—	—	—	—	—	—	—	—		
SB2A	87	114	62	72	66	98	68	91	73	51		
SB2B	70	79	64	61	46	74	54	68	57	54		
	+	+	+	+	+	+	+	+	+	+		
SB3A	51	8	3	5	4	2	116	7	3	7		
SB3B	75	7	2	3	2	1	45	3	3	2		
	+	—	—	—	—	—	+	—	—	—		
SB4A	7	102	83	73	2	5	6	76	69	51		
SB4B	5	104	68	63	2	5	6	190	137	130		
	—	+	+	+	—	—	—	+	+	+		
SB5A	5	6	4	2	2	2	8	7	5	6		
SB5B	10	27	8	6	3	3	5	25	21	11		
	—	—	—	—	—	—	—	—	—	—		
DR1	ND	ND	ND	ND	ND	ND	100	31	79	11		
SB*	2, 3	2, 4	2, 4	2, 4	2	2	2, 3	2, 4	2, 4	2, 4		

HLA haplotypes: *a*, A1 B8 DR3 SB4 *Glo**2; *b*, A30 B13 DR3 SB2 *Glo**2; *c*, A1 B8 DR3 SB2 *Glo**1; *d*, A2 B5 DR1 SB3 *Glo**1; *d/c*, A2 B5 DR1 SB2 *Glo**1. Results for expt 1 were obtained as described in Table 1 legend. In expt 2, lymphoblastoid cell lines were used as stimulators at 3×10^4 per well following treatment with mitomycin C (Sigma) at $80 \mu\text{g ml}^{-1}$ in RPMI 1640 for 30 min at 37°C . Absolute c.p.m. for control responses (100% reference) ranged between 13,272 and 30,837 c.p.m. in expt 1 and between 10,006 and 99,773 in expt 2. Results are tabulated and scored as in Table 1. Although the DR antigens are usually defined by serological techniques, a 'DR1-specific' PLT reagent was used as a cellular typing control in certain experiments. This reagent was generated using procedures similar to those for the SB-specific primed cells, but using lymphocytes from donors matched for all HLA antigens (including SB) but mismatched for DR1. Results of typing with this PLT correlate highly ($r = 0.83$) with serotyping for DR1. Family K was serotyped using antisera of the 7th US Workshop and additional DR typing was performed by the University of Minnesota Tissue Typing Laboratory using 118 well-defined antisera. ND, not determined.

* SB assignment.

(KAV3) with the maternal *d* haplotype (A2, B5, DR1) inherited SB2 but not SB3. These data can be explained by postulating a single recombination whereby sibling KAV3 inherited a recombinant maternal HLA haplotype, including the A2, B5, DR1 markers of the *d* haplotype but the SB2 marker of the *c* haplotype. The conclusion that sibling KAV3 has a SB/DR recombinant haplotype rests most heavily on the findings that: (1) the mother (KAV1) is indeed SB3-positive, (2) KAV3 is SB3-negative, and (3) KAV3 has inherited the maternal DR1 antigen. SB typing was repeated twice more, including a typing of lymphoblastoid B-cell lines derived from four family members (Table 2, expt 2). The results of these experiments confirm the SB typing of family members, particularly the determination that SB3 is present on maternal cells but absent from cells of sibling KAV3.

Although the serological assignment of DR1 in family members was unambiguous, as an additional control the cells were typed using a primed lymphocyte reagent which detects DR1 (or a specificity closely associated with it) (Table 2, expt 2). The results obtained with this PLT support the results of serotyping in this family—that mother and sibling 3 both express the DR1 antigen, but the other family members do not. Thus, using only cellular reagents, we can conclude that a maternal recombination has separated two loci, SB3 and DR1, that code for antigens which induce strong secondary proliferation.

These two families demonstrate that SB can segregate separately from DR. Mapping of the SB locus also requires information on its position relative to HLA-B and GLO. The data from family Fa map SB1 centromeric to DR but do not map it relative to GLO; none of the other families tested maps SB1 relative to GLO. The data from family Kav map SB2 and SB3 outside the region between HLA-A and HLA-DR; however, because GLO is not informative, it is not possible to ascertain from this recombination whether SB2 maps centromeric to HLA-DR or telomeric to HLA-A. Fortunately, results in other recombinant families allow unequivocal mapping of SB2 between DR and GLO (Table 3). The SB2 allele is mapped telomeric to GLO (on the DR side) by recombinations in both family Po and family H. In family Po, recombinant sibling P5 inherited the *GLO**2 allele but not the SB2 allele from the *d* haplotype; instead he acquired the SB blank allele (and A, B, C, DR) from the *c* haplotype. In family H, sibling H4 has acquired the *GLO**2 allele from the *b* haplotype, but has acquired the SB2 allele (along with A, B and DR) from the *a* haplotype. The results in family M map SB2 centromeric to HLA-A. The informative donor (M5) has a maternal HLA-A/HLA-C cross-over; she has acquired the SB2 (together with Cw3 B15) from the paternal *a* haplotype but the Aw30 from the paternal *b* haplotype. The combined results from the families places the gene encoding SB2 in the segment between HLA-DR and GLO; this is the only possible map location because SB2 maps centromeric to HLA-A (family M), outside the HLA-A to HLA-DR segment (family Kav) and telomeric to GLO (families H and Po).

The mapping of SB2 between DR and GLO agrees with the available mapping data for all the other SB antigens¹⁶ and unpublished observations. As there is strong evidence that the SB antigens may be alleles at a single locus¹⁶⁻¹⁸, the simplest hypothesis is that there is a single SB gene which maps in this location (although it is possible that they are pseudo-allelic products of more than one gene). The gene products of this locus are identified by their capacity to induce allogeneic cellular immune responses *in vitro*¹⁶⁻¹⁸; as this is a cardinal feature of histocompatibility antigens, the SB locus seems to be a histocompatibility gene.

Although the number of recombinations between DR and SB are too few to estimate precisely the map distance between them, the available data suggest that the SB locus may extend the known limits of the HLA region by as much as 1–2 centimorgans. In the 21 families studied, two definite SB/DR recombinations have been observed (family Kav and family Fa); in addition, one family²⁰ has a SB/HLA-B recombination, but it cannot be established whether the recombination is between SB and DR or DR and B, because the recombining haplotypes share DR/Dw4. After exclusion of family Fa (as the recombination was detected on a haplotype selected for known recombination), one (or possibly two) DR/SB recombinations have been observed in 75 'random' informative meioses. An additional crude index is that one of the four informative DR/GLO haplotypes mapped SB on the GLO side, whereas three mapped it on the DR side in the 5-centimorgan interval between DR and GLO.

It is possible that DR and SB are products of the same gene, and that they can segregate separately when intragenic recombination occurs. However, the discovery of two (or possibly three) SB/DR recombinations in these relatively few families makes intragenic recombination an unlikely explanation. Furthermore, selective loss of expression of HLA-DR but not SB in γ -ray-induced HLA-mutant cell lines¹⁹ is also unlikely to be due to intragenic recombination.

Many laboratories have reported PLT detection of new antigens. If all these antigens are products of different genes, that would imply great genetic complexity. Instead, preliminary evidence indicates that three laboratories have independently recognized similar antigens. Our study suggests that the GI antigen defined by Mawas *et al.*^{10,11} is similar to SB1 because both are encoded by genes which map centromeric to DR, and

Table 3 Mapping of SB2 between GLO and HLA-A in recombinant families

	Family Po					Family H				Family M				
	P1 <i>ab</i>	P2 <i>cd</i>	P3 <i>bd</i>	P4 <i>ac</i>	P5 <i>bd/c</i>	H1 <i>cd</i>	H2 <i>bd</i>	H3 <i>ac</i>	H4 <i>b/ac</i>	M1 <i>ab</i>	M2 <i>cd</i>	M3 <i>ad</i>	M4 <i>bc</i>	M5 <i>b/ad</i>
PLT reagents														
SB1A	6	9	4	2	3	86	84	11	14	14	8	7	14	17
SB1B	3	3	3	1	2	89	84	8	8	7	4	4	7	7
	—	—	—	—	—	+	+	—	—	—	—	—	—	—
SB2A	4	74	108	5	4	2	3	109	68	156	4	132	7	156
SB2B	4	82	83	9	24	3	2	101	62	140	5	87	5	124
	—	+	+	—	—	—	—	+	+	+	—	+	—	+
SB3A	6	3	5	2	10	3	4	8	8	6	4	5	4	7
SB3B	3	2	5	2	3	8	4	2	3	5	4	4	7	7
	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SB4A	92	5	78	64	104	109	16	121	89	35	26	38	23	46
SB4B	113	7	81	74	97	111	3	143	87	57	61	66	58	65
	+	—	+	+	+	+	—	+	+	+	+	+	+	+
SB5A	4	6	2	2	4	16	12	9	5	5	4	5	6	7
SB5B	7	7	5	6	11	10	6	15	12	14	5	6	12	12
	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SB assignment	4	2	2, 4	4	4	1, 4	1	2, 4	2, 4	2, 4	4	2, 4	4	2, 4

Haplotypes, in families Po, H and M respectively, are the following. *a*: A2 B5 Cw- DR2 SB4 GLO2; Aw34 B17 Cw- DR2 SB2 GLO1; A2 B15 Cw3 SB2. *b*: A25 B5 Cw2 DR5 SB4 GLO2; Aw33 Bw35 Cw2 DR7 SB(1) GLO2; Aw30 Bw50 Cw6 SB4. *c*: A1 B40 Cw3 DR7 SB- GLO1; A2 B8 Cw- DR3 SB4 GLO1; A3 Bw44 Cw3 SB4. *d*: Aw24 Bw35 Cw4 DR5 SB2 GLO2; A3 B18 Cw5 DR- SB1 GLO1; A3 Bw35 Cw4 SB4. *d/c* (Po): A1 B40 Cw3 DR7 SB-/GLO2. *b/a* (H): Aw34 B17 Cw- DR2 SB2 GLO2. *a/b* (M): Aw30/Bw15 Cw3 SB2. Results were obtained, tabulated and scored as described in Table 1 legend. Absolute c.p.m. for control responses in these experiments ranged between 11,218 and 33,959. For the *b* haplotype in family H, the SB antigen could be either SB1 or SB blank because it is identifiable in its entirety only in donor H2; this uncertainty does not interfere with the information regarding SB2 derived from the recombination.

both are present on the same two independent haplotypes in this family. Termijtelen *et al.*²¹ have defined a PLT specificity, PL3A, which is indistinguishable from SB1 in a population study ($r = 1.0$, $P = 10^{-6}$; A. Termijtelen and S.S., unpublished observations). The simplest interpretation of these data may be that these three antigens are identical, and that the gene encoding them is the one whose gene product fairly consistently dominates the secondary proliferative response among donors matched for HLA-A, B, C, D and DR.

Thus, SB is a new histocompatibility locus which is outside the previously well accepted boundaries of the HLA region. Data from recombinant families formally map SB2 between DR and GLO and, by inference, the gene encoding the four other SB antigens. The relevance of this new locus to transplantation, immune regulation and disease susceptibility is being investigated.

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SB: a new HLA-linked human histocompatibility gene defined using HLA-mutant cell lines

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Although the genes of the major histocompatibility complex (MHC) are tightly linked, four loci (HLA-A, B, C and DR) have been defined using rare families in which antigenic specificities segregated from each other. As an alternative approach for defining loci, Kavathas *et al.*¹ selected HLA-mutant lymphoblastoid cell lines (LCLs) that had lost expression of specific genetic markers. An HLA-GLO heterozygous LCL, LCL-721, was mutagenized with ionizing radiation which is known to induce multigenic deletions²; variants no longer expressing a specific HLA antigen were selected. While some mutants lost expression of only the antigen selected against, most simultaneously lost expression of several identifiable *cis*-linked gene products. Many of the multiple-loss mutants resulted from chromosome rearrangements, especially deletions¹. Five new secondary B-cell (SB) antigens have recently been defined^{3,4}, which evoke strong secondary allogeneic proliferative and cytotoxic responses. Because they resemble the HLA-DR antigens both functionally and genetically, it was not known whether they were coded for by a gene distinct from the gene(s) encoding HLA-DR. We have now characterized LCL-721 mutants for expression of both HLA-DR and SB. Of 20 lines which had lost expression of the HLA-A, -B and -DR antigens from one haplotype, four retained expression of the *cis*-linked SB antigen. One of these mutants had a visible deletion that included the region coding for HLA on the short arm of one chromosome 6. Because the *cis*-linked SB antigen continued to be expressed, the gene coding for SB antigens must be different from that coding for HLA-DR antigens.

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Table 1 SB typing of mutant lymphoblastoid cell lines

LCL clone	DR status	GLO status	Stimulation of PLT (c.p.m. $\times 10^{-3}$)					% Expression of		
			SB2A	SB2B	SB4A	SB4B	DR1	SB2	SB4	DR1
Experiment 1										
Parent										
721	1, 3	1, 2	25.4	44.9	40.1	36.2	47.1	100	100	100
Mutant clones										
1	1	1	20.2	46.0	7.8	5.0	45.8	94	17	97
19*	1	1, 2	33.1	49.9	41.2	42.5	43.5	118	110	92
22	1	1, 2	27.8	42.1	1.6	0.9	40.0	99	3	85
31	1	1, 2	32.0	48.2	5.0	5.2	48.6	114	13	103
34	1	1, 2	24.8	39.2	7.1	5.7	45.4	91	17	96
38	1	1	20.7	36.6	5.5	5.9	40.5	82	15	86
40	1	1, 2	19.3	32.4	4.9	3.4	40.8	74	11	87
51*	3	1, 2	19.7	40.8	32.6	33.2	2.4	86	86	5
52*	3	1, 2	8.6	31.4	19.6	23.1	1.2	57	56	3
Control LCL										
H9 (SB1, 2)			20.8	44.8	15.2	2.7		93	23	
W7 (SB1, 4)			0.6	1.4	31.5	51.1		4	108	
Experiment 2										
Parent										
721	1, 3	1, 2	7.0	13.1	9.8	13.8		100	100	
Mutant clones										
1	1	1	8.5	13.4	2.4	3.2		110	24	
3	1	1, 2	6.0	13.2	1.0	1.4		96	10	
13	1	1, 2	6.3	11.7	0.9	0.6		90	6	
22	1	1, 2	8.4	14.1	2.2	2.0		112	18	
38	1	1	10.0	13.6	3.1	2.1		118	22	
51*	3	1, 2	3.4	10.1	6.1	9.5		68	66	
52*	3	1, 2	5.0	12.0	7.9	11.4		85	82	
54*	3	1, 2	1.9	7.6	3.9	8.0		48	50	
56	3	1, 2	0.5	0.6	10.3	12.9		6	98	
Control mutant (B8 loss only)										
21	1, 3	1, 2	7.2	13.4	10.5	13.1		103	100	

The method for generating SB-specific primed cells and the technique of primed lymphocyte typing has been described elsewhere⁵. LCLs were treated with 80 $\mu\text{g ml}^{-1}$ mitomycin C (Sigma) in RPMI 1640 for 30 min at 37 °C, then washed three times. In experiment 1, LCLs were at a concentration of 0.3×10^5 per well and in experiment 2 at 0.1×10^5 per well. Per cent expression is calculated as (c.p.m. incorporated by the relevant responding cells when stimulated by the mutant line)/(c.p.m. incorporated when stimulated by the parent line); the mean of the two SB-specific PLTs was used. Glyoxalase I analysis and DR typing were performed as described previously¹.

* Mutants showing separation of DR and SB expression.

We used LCL-721 established from lymphocytes of donor KAV3, which had the genotype HLA-A1, -B8, -DR3, GLO*2 and HLA-A2, -B5, -DR1, GLO*1. Mutants that had lost expression of DR3 were derived after γ -ray irradiation and selection with HLA-B8 antiserum and rabbit complement, and mutants that had lost expression of DR1 were derived after irradiation and selection with HLA-B5 antiserum or HLA-A2 antiserum and complement.

SB-typing reagents were generated by sensitizing peripheral blood lymphocytes in a primary culture to cells matched for HLA-A, -B, -C, -DR, MB but mismatched for SB⁵. The primed cells were restimulated with the same stimulating cells and then frozen. Cells to be SB typed were used as restimulating cells. SB typing of LCL-721 of donor KAV3 and of her family showed that LCL-721 expressed SB2 linked to DR1 and SB4 linked to DR3. The SB2, DR1 haplotype inherited by individual KAV3 from the mother appears to be a recombinant haplotype because the mother expressed SB2 on one haplotype and DR1 on the other haplotype⁴.

In experiment 1 seven mutant clones that had lost expression of DR3, B8 and A1 were tested (Table 1); six of these had a marked diminution in the SB4-specific proliferative response (3–17% of control) but one, clone 721.19, retained normal ability to evoke SB4-specific stimulation (110% of control value). All seven mutants evoked a strong SB2-specific proliferative response (74–118% of control). Two mutant clones that had lost serologically detectable expressions of DR1, B5 and A2 were tested in the same experiment; both retained their capacity to stimulate SB4- and SB2-specific proliferative responses. Although the ability of 721.52 to stimulate SB2-specific responses is reduced compared with controls (57% of control), this is a nonspecific effect which is also seen with SB4-specific responses (56% of control).

Although the DR antigens are usually defined by serological techniques, a DR1-specific primed lymphocyte typing⁶ reagent (PLT) was used as a cellular typing control in some experiments. This reagent was generated using procedures similar to those for the SB-specific primed cells, but lymphocytes from donors matched for all HLA antigens (including SB) but mismatched for DR1 were used. Results of typing with this PLT correlated highly ($R = 0.83$) with serotyping for DR1. Mutants 721.51 and 721.52 which according to serological analysis, had lost expression of DR1, also demonstrated loss of DR1 (or a specificity highly associated with it) when tested with the cellular reagent (Table 1).

Additional SB typing of mutant clones is shown in Table 1, experiment 2. In this experiment, all the DR3-loss mutants tested had lost expression of the *cis*-linked SB4 antigen, whereas only one of the four DR1-loss mutants, LCL 721.56, had lost expression of the *cis*-linked SB2 antigen. Overall, studies of 20 mutants showed that one of 15 DR3-loss mutants retained expression of SB4 and three of five DR1-loss mutants retained expression of SB2. None of the four DR3-loss or one DR1-loss mutants which had also lost expression of the *cis*-linked GLO allele expressed the *cis*-linked SB antigen.

Two of the mutants in which expression of DR and SB had been separated were further analysed to determine whether the loss of expression of HLA-DR resulted from deletion of genetic material. The complete karyotype of mutant 721.19 (which had lost expression of A1, B8 and DR3 but not SB4 or GLO*2) has been published¹. An interstitial deletion was observed in 6p where the HLA genes have been mapped⁷. No other chromosome abnormality was detected. DNA hybridization studies also show that mutant 721.52 resulted from deletion of genetic material: DNA from this mutant has greatly reduced ability (compared with LCL-721) to reassociate with a radio-

active probe prepared from an HLA-specific (class I) cDNA clone (ref. 8 and H. Orr, personal communication); this indicates that the mutant has lost genetic material which is homologous to the probe. The mutants that express both SB2 and SB4 but have apparently lost expression of an HLA-DR antigen because of loss of genetic material provide evidence that SB and DR are encoded by distinct genes. As the region between *HLA-DR* and *HLA-A* appears to be lost and as *SB* maps between *GLO* and *HLA-B*³, these results indicate that *HLA-SB* maps centromeric to *HLA-DR*.

The proportion of DR3-loss mutants which express the *cis*-linked SB4 antigen (1/15) is less than the proportion of DR1-loss mutants which express the *cis*-linked SB2 antigen (3/5). This difference could be due to chance or could have a biological basis. Further studies of segregation between *SB* and *DR* in LCL-721 and other mutant lymphoblastoid lines should help define the physical and functional relationships between *SB* and other loci.

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Persistence of non-integrated viral DNA in bovine cells transformed *in vitro* by bovine papillomavirus type 2

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Papillomaviruses induce benign tumours (warts) in their natural hosts. Several bovine papillomaviruses (BPV 1-5) have been described¹⁻⁵. BPV 2 represents the virus isolated from classical cutaneous fibropapillomas found on the neck and shoulders of cattle⁵. In heterologous hosts, including the hamster and horse, it induces fibrosarcomas, sarcomas and fibromas, none of which possess virus particles but all of which contain viral DNA⁶⁻⁸. Both BPV 2 and its DNA induce cellular transformation *in vitro*⁹⁻¹⁶, although the presence of viral DNA in such transformed bovine cells has so far not been reported. Here we demonstrate the presence and maintenance of BPV 2 DNA sequences in bovine cells transformed *in vitro* by BPV 2.

BPV 2 was isolated from single-case fibropapillomas of the neck and purified as described previously¹⁷. Tissue cultures were established from bovine conjunctiva and palate, and primary cell cultures passaged several times before virus infection. After BPV 2 infection¹³, three independently derived cell lines were cultured *in vitro* to over 40 passages. Two of these lines arose from infection of bovine conjunctiva at the 12th and 16th passage and are henceforth designated 12CON/BPV 2 and 16CON/BPV 2. The third line originated from infection of

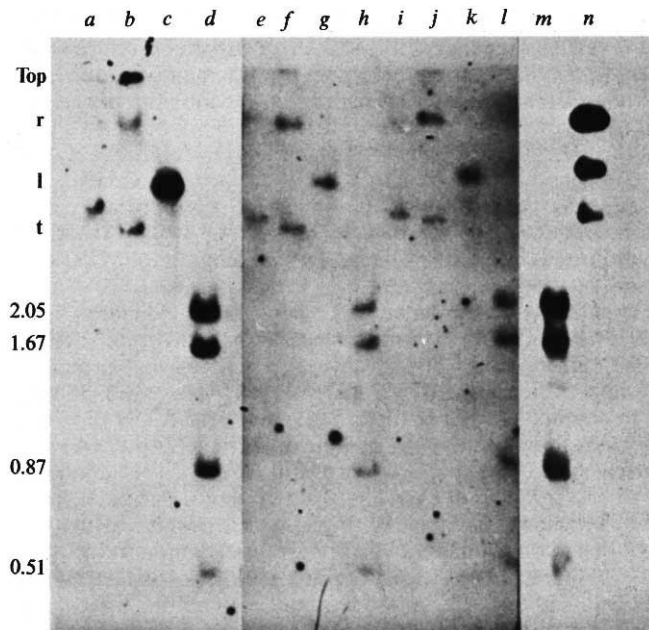


Fig. 1 Autoradiograms of BPV 2 DNA sequences in transformed bovine cell lines. *a-d*, 16CON/BPV 2; *e-h*, 12CON/BPV 2; *i-l*, 17PAL/BPV 2; *m, n*, BPV 2(1 ng). *a, e, i, n*, Unrestricted DNA; *b, f, j*, *EcoRI*-restricted DNA; *c, g, k*, *HindIII*-restricted DNA; *d, h, l, m*, *HindII*-restricted DNA. Purified cellular DNA (10 µg) was digested with individual restriction enzymes in buffers recommended by Boehringer. After 16 h at 37°C, cleaved DNA was separated out on a 1% agarose gel in E buffer²² at 5 V cm⁻¹. The resulting fragments were transferred to nitrocellulose sheets¹⁸ and hybridized to a nick-translated²³ ³²P-labelled DNA probe (specific activity 5 × 10⁷ c.p.m. per µg) in 6 × SSC, 10 × Denhardt's²⁴ solution containing 50 µg ml⁻¹ calf testis DNA, 10 µg ml⁻¹ poly(A), 0.1% SDS and 10 µg ml⁻¹ yeast tRNA. After hybridization at 70°C for 3 days at a DNA probe concentration of 0.05 µg ml⁻¹, the sheets were extensively washed in 1 × SSC at 60°C, dried and exposed to flash-activated X-ray film (Kodak X-omat H) at -70°C overnight²⁵. As a template for nick-translation, BPV 2 DNA was purified by EtBr/CsCl gradient centrifugation and, as will be described elsewhere (M.S.C. *et al.*, in preparation), molecularly cloned in pAT 153, a derivative of pBR322 plasmid vector. The molecular weights (× 10⁻⁶) of *HindIII*-digested BPV 2 DNA have been included as references for DNA fragment length. The sensitivity of these experiments was between one and two viral genome equivalents, based on reconstruction experiments. *r*, Relaxed circles; *l*, linear molecules; *t*, twisted circles.

bovine palate at the 17th passage and is henceforth designated 17PAL/BPV 2. The properties of the cell lines will be described elsewhere but all three exhibited a phenotype with features characteristic of cellular transformation including loss of contact inhibition, growth in agarose, tumour production in heterologous hosts and altered cell morphology compared with normal cells. Some of these characteristics have been described previously¹³. High-molecular weight cellular DNA was prepared from each cell line at various passages and cleaved with the restriction enzymes *EcoRI*, *HindIII* and *HindII*. The cleavage products were separated according to their molecular weights by agarose-gel electrophoresis and the resulting fragments transferred to nitrocellulose¹⁸. DNA sequences complementary to BPV 2 DNA were detected by hybridizing a BPV 2 DNA probe radioactively labelled with ³²P to the nitrocellulose sheets. The template for the probe represented the BPV2 genome ligated to plasmid vector pAT153 and molecularly cloned in *Escherichia coli* (M.S.C. *et al.*, in preparation). The cellular DNA fragments which hybridized to BPV 2 DNA are shown in Fig. 1. In all three cell lines, BPV 2 DNA sequences were detected at electrophoretic mobilities corresponding to the fragments expected from cleaving BPV 2 DNA with the same enzymes. Unrestricted DNA produced fragments which co-migrated with the two molecular forms of BPV 2 DNA: forms I and II, corresponding to twisted circular and relaxed circular molecules, respectively. *EcoRI* does not cleave BPV 2 DNA, *HindIII* cuts once (linear form of molecular weight 5 × 10⁶), and *HindII* cuts four times, producing fragments with molecular weights of 2.05,

1.67, 0.87 and 0.51×10^6 (refs 1, 4). Cleaving the DNA of the three cell lines independently with these enzymes produce DNA fragments which co-migrated with the fragments produced by cleaving BPV 2 DNA. The slightly slower mobility of the two forms of viral DNA in the unrestricted samples compared with the *EcoRI*-restricted ones and the marker DNA is probably due to the generally slowed migration of DNA fragments when a large amount of high-molecular weight DNA is present. There is no hybridization of the BPV 2 DNA probe to DNA derived from normal calf testis, spleen, liver, kidney, brain or salivary gland data (not shown).

To estimate the amount of BPV DNA in individual cell lines, DNA was loaded on to nitrocellulose disks and hybridized to the BPV 2 DNA probe¹⁹ (see Table 1). Each cell line contained multiple copies of the BPV 2 genome, ranging over 33–55 viral genome equivalents per diploid quantity of cell DNA. *In situ* hybridization with BPV 2 complementary RNA(cRNA) was performed to determine whether viral DNA was accumulating in all or a few cells of each line. Table 1 shows that in each cell line a very low percentage of the cells were positive by this test and therefore contained disproportionate amounts of viral DNA. Figure 2 shows such a positive cell in the 16CON/BPV 2 cell line. Although the autoradiograph was exposed for 1 week, the same distribution of positive cells was observed after 2 months' exposure (data not shown).

The cell lines were also examined by various methods for the presence of virus particles or antigens. An extensive electron microscopy search of 1,200 nuclear profiles for each cell line was carried out. Spent culture medium was bulked and pelleted: pellets were subjected to velocity gradient and isopycnic centrifugation and appropriate bands were examined by negative staining. Cells were examined immunohistochemically by a peroxidase-anti-peroxidase (PAP) technique using a potent antiserum to SDS-disrupted 'virions' prepared in rabbits. This antiserum has a titre of 1:20,000 against BPV 2 virions in skin papillomas. However, no evidence of virus or viral antigens was found.

These results are interesting for several reasons. First, this constitutes the first detection of papillomavirus DNA in *in vitro* transformed cells of the natural host. Second, while we cannot completely rule out the existence of a few subgenomic viral DNA sequences integrated into the host genome, it is clear that the most viral DNA sequences exist in free form. This viral DNA most probably represents an episomal state as we have consistently failed to detect either virus antigens or virus particles in these cell lines. The few cells containing high amounts of viral DNA probably result from the very rare induction of disproportionate viral DNA synthesis, and their low frequency suggests that they are highly unlikely to contribute significantly

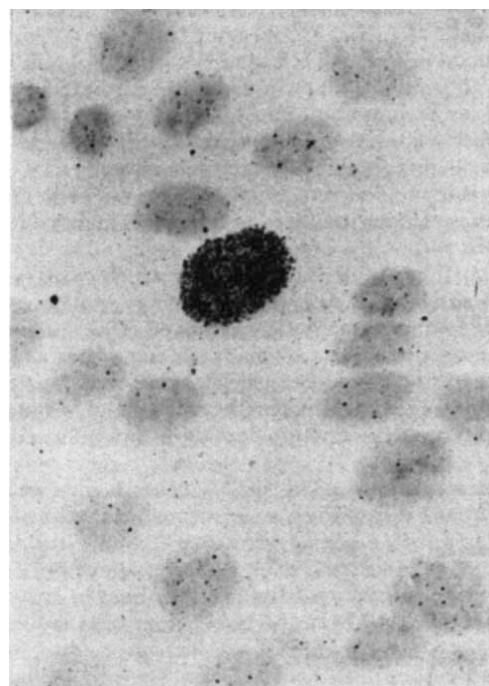


Fig. 2 *In situ* hybridization of BPV 2 cRNA to BPV 2-transformed cells. BPV 2 cRNA was synthesized using *E. coli* RNA polymerase and all four ribonucleotide triphosphates labelled with ³H according to previously described procedures²⁶; the specific activity of cRNA was 2×10^7 c.p.m. per μ g. Cells were fixed at the 24th passage in culture in methanol/acetic acid (3:1), denatured in 0.05 M NaOH, and hybridized with $3 \text{ ng } \mu\text{l}^{-1}$ ³H-labelled BPV 2 cRNA for 16 h at 60 °C in $3 \times \text{SSC}$. After hybridization, the cells were treated with $30 \mu\text{g ml}^{-1}$ pancreatic RNase at 37 °C for 30 min extensively washed with $2 \times \text{SSC}$ and finally air dried. Autoradiography was performed using Ilford K2 emulsion and the autoradiographs exposed at 4 °C for 1 week. After developing, fixing and washing with distilled water, cells were stained in 6% Giemsa in phosphate buffer as described elsewhere²⁶.

in the analysis of viral DNA detected here. In addition, further passaging in culture led to a disappearance of these cells, while the viral genome number remained at ~30–50 copies (not shown). We are now analysing cell clones to determine whether the viral genomes are equally distributed throughout the cell population.

Recently there have been reports of non-integrated papillomavirus DNA in tumour cells derived from natural infection with papillomavirus^{8,20,21}. If non-integration of papillomavirus DNA into the host cell genome becomes a general finding then the comparison with other members of the Papovavirus family, where integration of viral DNA is usual, could be highly significant in terms of the analysis of oncogenic virus-cell interactions.

Finally, our results underline further the difficulty in establishing a suitable *in vitro* tissue culture system to propagate papillomavirus; thus far the keratinizing cells of the dysplastic epithelium of naturally occurring tumours represent the only site in which virus particles have been identified.

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Note added in proof: Law and colleagues²⁷ have recently detected non-integrated viral DNA in mouse cells transformed *in vitro* by BPV 1.

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Table 1 Detection of BPV 2 DNA in transformed cells

Cell DNA*	c.p.m. ³² P hybridized†	Average viral genome equivalents	% Cells positive by <i>in situ</i> hybridization
12CON/BPV 2	2,022 ± 80	55	0.02
16CON/BPV 2	1,209 ± 55	33	0.5
17PAL/BPV 2	1,397 ± 35	38	0.1
Calf testis	155 ± 15	<1	—
Blank filter	132 ± 12	—	—
2 ng BPV2 + calf	1,829 ± 14	50	—
5 ng BPV2 testis	4,599 ± 37	125	—

20 μ g DNA per filter were hybridized to $0.1 \mu\text{g ml}^{-1}$ [α -³²P]BPV 2 DNA probe in $6 \times \text{SSC}$, $10 \times \text{Denhardt's}$ containing $50 \mu\text{g ml}^{-1}$ calf testis DNA, $10 \mu\text{g ml}^{-1}$ poly(A), $10 \mu\text{g ml}^{-1}$ yeast tRNA, 0.1% SDS at 70 °C for 20 h. The average viral genome equivalents per diploid quantity of host DNA were calculated based on the 5×10^6 MW (viral) and 3×10^{12} MW (host) DNA. See Fig. 2 legend for the conditions of *in situ* hybridization.

* 24th passage in cell culture.

† Average of three separate determinations.

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Evolution of rRNA and origin of mitochondria

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The origin of mitochondria has remained a matter of discussion and speculation since Altmann first proposed the endosymbiont hypothesis in 1890¹. Mitochondria may have arisen by the invasion of aerobic^{2,3} or anaerobic photosynthetic⁴ bacteria into ancestral protokaryotic cells, or in a non-symbiotic fashion by compartmentalization of episomal DNA^{5,6}. We have now tested these hypotheses by comparing nucleotide sequences of small ribosomal subunit RNA (S-rRNA) genes from mitochondria of *Aspergillus nidulans*, yeast⁷, man⁸ and mouse⁹, from nuclei of yeast¹⁰ and animals^{11–13}, and from *Escherichia coli*¹⁴ and maize chloroplasts¹⁵. All rRNA molecules deduced from gene sequences share several regions of conserved primary and potential secondary structure. An evolutionary tree analysis of gene sequences supports the endosymbiotic eubacterial origin of fungal mitochondria and further suggests an independent bacterial origin of animal mitochondria.

Figure 1 compares the *A. nidulans* mitochondrial (mt) S-rRNA gene sequence¹⁶ with all published sequences. The recently determined sequence of the *Xenopus laevis* nuclear gene (not included in the compilation) shows extensive homology to the yeast nuclear sequence¹². It is clear that all S-RNA molecules share several sequence-conserved regions around matrix positions 570, 990, 1,120, 1,680 and 1,810. The two 3'-terminal regions contain sequences of 18 and 15 nucleotides, respectively, common to all species with the exception of

the two fungal mitochondrial sequences. The 3'-terminal sequence -CCUCCU- of bacterial and chloroplast S-rRNA interacting with mRNA during polypeptide chain initiation¹⁷ is missing in all nuclear and mitochondrial sequences, indicating that a bacterium-like mechanism of mRNA recognition does not operate in mitochondria.

In a second class of regions the bacterial, chloroplastic and fungal mitochondrial sequences are more closely related to each other than to the nuclear sequences (5'-terminal region between positions 10 and 70, and regions around positions 110, 320, 380 and 1,600). The third and most abundant class of S-rRNA gene regions does not show significant homology between the various species, and most of the deletions are found in such variable regions. The number of deleted nucleotides relative to the nuclear sequence increases in the order: *E. coli*, chloroplasts, fungal and mammalian mitochondria.

Figure 2 demonstrates that the *A. nidulans* mt S-rRNA molecule (as deduced from the DNA sequence) can be folded in almost exactly the same way as proposed for *E. coli* 16S rRNA¹⁸. Using the alignment of Fig. 1 we also obtained very similar structures for yeast cytosolic and human mt S-rRNA. The yeast 18S rRNA model differs in some details from that of Brimacombe¹⁸, which was based on incomplete sequence data. The product of the *X. laevis* gene¹¹ can be folded exactly like the yeast molecule.

The conservative features of all S-rRNA molecules (see Fig. 2) include six hairpin structures, seven long-range interactions and several single-stranded sequences (hairpin loops and stem-connecting loops). Almost all modified nucleotides of the *E. coli* molecule¹⁹ are located in this conserved core region, which may be involved in the essential functions of the small ribosomal subunit.

Figure 2 also shows that the yeast cytosolic molecule differs from the bacterial and mitochondrial species by several inserts of low potential secondary structure (see left half of the model), and that the two mitochondrial species of *A. nidulans* and human cells have progressively deleted peripheral structures of the bacterial molecule.

We have selected six relatively conserved and non-deleted regions of S-rRNA genes (Fig. 1, positions 6–70, 548–632, 1,083–1,213, 1,408–1,500, 1,619–1,700 and 1,789–1,853) for a phylogenetic tree analysis^{20,21}. Table 1 shows the combined difference matrix of all compared sequences and, separately for each region, the relative affinities between bacterial and organelle sequences. In all six regions of the S-rRNA genes the chloroplastic and mitochondrial sequences are more closely related to the bacterial than to the nuclear sequence, and the prokaryotic affinity decreases in the order: chloroplast, fungal mitochondria, mammalian mitochondria.

Partial sequence data of the *A. nidulans* nuclear S-rRNA gene (P. Borsuk, H.G.K. and H.K., unpublished) indicate that the average substitutional distance between yeast and *A. nidulans* nuclear sequences is at least three times less than the distance between the corresponding mitochondrial gene sequences, suggesting a higher rate of nucleotide substitution in fungal mitochondria. The difference between mitochondrial and nuclear rates of substitutions in rRNA genes is even higher in

Table 1 Difference matrix of S-rRNA gene regions

	Difference							Difference to yeast, n				
	(% of all positions in regions 1-6)							Difference to <i>E. coli</i>				
	<i>Xenopus</i> n	Yeast n	<i>E. coli</i>	Maize ch	<i>A. nidulans</i> mt	Yeast mt	Human mt	1	2	In region 3	4	5+6
Yeast, n	10.1											
<i>E. coli</i>	38.9	35.7										
Maize, ch	41.3	37.8	14.7					4.70	2.92	3.34	2.58	1.84
<i>A. nidulans</i> , mt	48.3	48.0	34.4	35.7				1.94	1.25	1.40	1.54	1.21
Yeast, mt	49.2	47.6	42.6	43.4	29.2			1.56	1.34	1.31	1.30	1.19
Human, mt	49.6	49.7	42.6	43.4	45.6	46.2		1.23	1.20	1.14	1.19	1.20
Mouse, mt	50.6	50.3	45.6	47.2	46.2	47.2	18.2	1.13	1.15	1.04	1.10	1.12

Genes from: n, nucleus; ch, chloroplast; mt, mitochondrion.

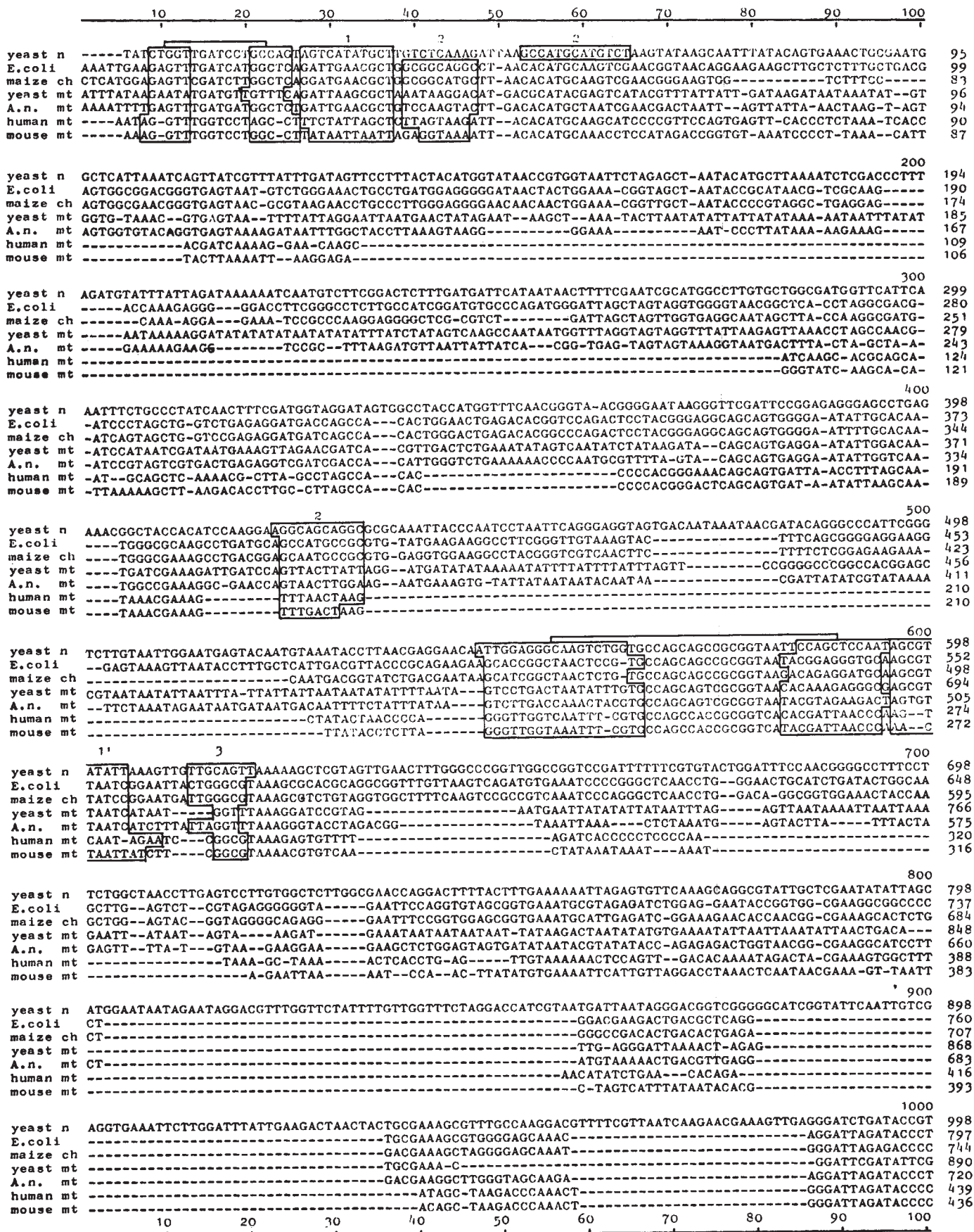


Fig. 1 Alignment of S-rRNA gene sequences from yeast (*Saccharomyces cerevisiae*) nuclei¹⁰ and mitochondria⁷, *E. coli* (rrnB)¹⁴, maize chloroplasts¹⁵ and from mitochondria of *A. nidulans*, human⁸ and mouse⁹ cells. The sequences start with the putative 5' termini of the gene products, and the nucleotide numbers of the individual sequences are indicated at the end of each line. The sequences were superimposed to maximal sequence homology by introducing a minimal number of gaps (dotted line). The boxed regions can form base-paired hairpin stems (brackets) or far-range interacting stems (numbers 1-7) in analogy to the secondary structure model for *E. coli* 16S rRNA¹⁸. Nucleotides 459-600 of the yeast mt sequence⁷ were omitted (between positions 502 and 503). The alignment includes two 3'-terminal sequences from *Drosophila melanogaster*¹² and *Bombyx mori*¹³ nuclei.

	10	20	30	40	50	60	70	80	90	100	
yeast n	CGTAGT-CTTAACCATAAACTATGCCGACTAGATCGGGTGGT--GTTTTTTTAAATGACCCACTCGGTACCTTACGAGAAATC-AAAGTCTTTG	1094									
E.coli	GGTAGT-CCACGCCGTAAACGATGTGCACTTG-----GAGGTT-TGTGCC-CT--TGAGGCGTGGC-TTCGGGAGCTAACCGGTTAAGTCGACGCGCT--G	883									
maize ch	AGTAGT-CCTAGCCGTAAACGATGGATACTAGGT-----GCT--GTGCGACT--CGACCCGTGCAGTGTCTAGCTAACCGGTTAAGTATCCGCT--G	830									
yeast mt	TGTAGTTTCTAGTAGTAAACTATGAATACAATTATTATTATAA-----TATATATTATATAAAATAAATAAATGAAATG-AAAGTATTCACCT--G	977									
A.n. mt	AGTAGT-CCAAGCAGACAAATGATGAATGTCTAGACTAGAAAAAGTCGTTAGACTATAAATTTAGTCTATAAATGAAAGTG-TAAGCATTCACCT--G	815									
human mt	ACTATG-CTTAGCCCTAAACC-TCAACAGTTAAATCAA-CA-----AACTGCTC-GCA-----AACTATTT-GCA-----	490									
mouse mt	ACTATG-CTTAGCCATAAACG-TAAATAATTAAATTTAACA-----AACTATTT-GCA-----	488									
yeast n	GGGGAGTATGGTGCAAAAGCTTAAAGGAATTGACGGAAGGGCACCA-CTAGGAGTGGAGCCTGGC-GCTAAATTTGACTCAACACGGGGAAC	1192									
E.coli	G--GAGTACGG-CGCAAGGTTAAACTCAAAATGAATTGACGCGGGCCCG-CA-CAAGCGGTGGAGCATGTGCTTTAATTCGATGCAACCGGAAGAAC	979									
maize ch	G--GAGTAC-GTTGCAAGATGAACTCAAAAGGAATTGACGCGGGCCCG-CA-CAAGCGGTGGAGCATGTGCTTTAATTCGATGCAACCGGAAGAAC	929									
yeast mt	--AAGAGTAC-GTTAGCAATAATGAACTCAAAACAATAGACGGTTACAGA-CT-TAAGCAGTGGAACTGTTATTTAATTCGATAATCCAGCACTAAT	1073									
A.n. mt	--AAGAGTAA-TATGCAACATATAAAGTAAATCATAGACGCTTTCTGA-GA-CGAGTGTGAATATGTTATTTAATTCGATGATCCGCAAAAC	911									
human mt	--GAAGAGTAC-GAGGCACAGCTTAAACTCAAGGACCTGGCGTGTCTTATATCCCTCTAGAGGAGCGTGTCTGTAAT-CGATAAACCCGATCAACC	587									
mouse mt	-GAGAAGTAC-TAGGCATAGCTTAAACTCAAGGAGCTTGGCGTACTTTATATCCATCTAGAGGAGCGTGTCTGTAAT-CGATAAACCCGCTCTACC	585									
yeast n	TCACAGGTCCAGACACAATAAGGA--TTGACAGAT-TGAGAGCTCTTTCTT--GATTTTGTGGGT--GGTGGTGCATGGCCGTTTCTCAGTTGGTGGAG	1285									
E.coli	TTACCTGGTCTTGACAT-CCACGGAAGTTTTCAGAGATGAGAA--GTGCCCTCGGGAACCGTGAGACAGGTGCTGCATGCTGT-CGTACGCTCGTCTTG	1076									
maize ch	TTACAGGGCTTGACATGCGCC-GAA-TCCTCTTGAAGACAGAGGGGTGCCCTCGGGAACCGGACACAGGTGGTGCATGCTGT-CGTACGCTCGTGGCG	1026									
yeast mt	TTACCATATTTTGA-ATATTATAATAATTATTATAATTATTATTT-----ACAGCGCTTACATTTGTTG-CTTTAGTTCGTCGTG	1152									
A.n. mt	TTACCAAGTCTGA-ATATTTT-----ACAAGCGCTGCAGGCTGT-TTTAGTTAATGTGCG	966									
human mt	TCACGACCTCTT-----	600									
mouse mt	TCACCATCTCTTGCTAA-----	602									
yeast n	TGATTTGCTGTCTTAATTTGCG-ATAAC-GAAGCAGAC-CTTAACCTAC-TAAATAGTGGTGTAGCAATTGCTGGTTATCCACTTCTTAGAGGGAGCATC	1381									
E.coli	TGAAATGTTGGGTTAAGTCCCGC-AAC-GAGCGCAACCCCTATCTTTGTTGCCAGCGGTCCGGCCGGGAACCTCAAGGAGACTGCC--AGTGATAA--	1169									
maize ch	TAAAGTGTGGGTTAAGTCTCGC-AAC-GAGCGCAACCCCTGCTGTTTAGTTGCCACTATG--AGTTTGAACCCCTGAACAGACCGCC--GGTGTAA--	1117									
yeast mt	CAAAAGTTTATGATTAAATGTGCATAAAGGACCAAACTCC-TAATGATCCTT--ATAATATGGTAAATAGAGCTGTATAATAA-AATGATAAATAAT	1216									
A.n. mt	TGAGATCT--GGTTAAGTC-CTTTAATTAACGAAACCCCTCA-CTTTATTGCA-----TTTATAAAGTTGATCGCC--TTTAT--	1039									
human mt	-----CTCAGCCTATATACCGCC--ATCTTCAG--	626									
mouse mt	-----TTCAGCCTATATACCGCC--ATCTTCAG--	628									
yeast n	GGTTTCAAGCCGATGGAAGTTTGAGG-CAATAACAGGTCTGTGATGCCC-TTAGAACGTTCTGGCGCCGCTACACTGACGGAGCCAGCGAGTC	1479									
E.coli	-----ACTGGAGGAAG-GTGGGGATGACGTCAAGTCA-TCATGCCCTT--ACGACAGGCTACACAGCTGTACAAT--GGCGCATACAAAGA	1253									
maize ch	-----GCCGGAGGAAG-GAGAGGATGAGGCCAAGTCA-CATGCCCTT--ATGCCCTGGCGACACAGCTGTACAATG--GGCGGG-ACAAAG	1200									
yeast mt	-----TTAATATAAAG-AAAGGAATTAAGACAAATCA-TAATGATCCTT--ATAATATGGTAAATAGAGCTGTATAATAA-AATGATAAATAAT	1302									
A.n. mt	-----ATTGGTTAGATAATAGGGATTAAGACAAAGTCA-TCATGG-CCTTA-ATACTGTGGCTATAGAGCTGTGCACA-TA--TGCCCTTACAAAG	1124									
human mt	-----CAACCCCTGAT-GAAGGCTACAAAGTAAGCGC-AAGTAC-CCACGTAAAGACGTTAGGTCAAGGGTTAGCCCA-TGA-GGTGGCAAGAAATG	713									
mouse mt	-----CAACCCCTAAA--AAGGTATTAAAGTAAGCAA--AGAATCAACATAAAGCGTTAGGTCAAGGGTTAGCCCA-TGA-AATGGGAAGAAATG	715									
yeast n	-----TAACCTTGGCCGAGAGGTCTTGGTAACTCTGTGAAACTCCGTCGTGCTGGGGATAGAGCATTGTAAAT	1546									
E.coli	-----GAAGCGACCTCGCGAGAGCAAGCGGA-CCTCATAAAGTCCGTCGTAGTCCGGATTGGAGTCTGCAAC	1319									
maize ch	-----GTCGGCATCTCGCGAGGGTGAGCTAA-CTCCAAAAC-CGTCCTCAGTCCGGATTGCGAGCTGCAAC	1267									
yeast mt	TATATAAATATATTTAATTATATTTAATTAATAAATAAAGATTTTAAATGTTTATTTATATATTAATTAATGAATTATAATCTGAAAT	1403									
A.n. mt	-----GATGGCATTTTGAAATTTAGCTAATCCCCAAAAA-AGGATATAATATGAGTTGATGCTGTAACT	1190									
human mt	-----GGCTACATTTTCTA-----CCCCAGAAAA-----CTAC-TATAGCCCTTATGAAA	757									
mouse mt	-----GGCTACATTTTCTT-----ATAAA-AGAAACA-----TTAC-TATACCCCTTATGAAA	760									
D.m. n	ATTCGAGTAAG-TGTGAGTCATTAAG-TCCGATTGATTAGTCCCTTGGCCGTTT-AC-ACACCGCCCGTCCGTACTACT	1700									
B.m. n	ATTCGAGTAAG-CGCGAGTCA-TAAGC-TGCGGTTGATTAGTCCCTTGGCCGTTTGAACACACCGCCCGTCCGTACTACT										
yeast n	TATTGCTCTTCAACGAGGAATTCCTAGTAAG-CGCAAGTCA-TCAGC-TGCGGTTGATTAGTCCCTTGGCCGTTT-AC-ACACCGCCCGTCCGTACTACT	1641									
E.coli	TCGACTCATGAAGTCGGAATCGCTAGTAAT-CGTGCATCAG-AATG-CCACGGTGAATAGTTCCTCGGCCCTTGT-AC-ACACCGCCCGTCAAC-ACCAT	1413									
maize ch	TCGCGCTGCATGAAGCAGGAATCGCTAGTAAT-CGCGGCTCAGCCATA-CGGCGGCAATCGTTCGCGGCCCTTGT-AC-ACACCGCCCGTCAAC-ACTAT	1360									
yeast mt	TCCATTATATGAAAAAGAAATTCCTAGTAATACGTAAATTTAGT-ATG-ITACCGTGAATATTCTAA-CTGTTTCCG-ACTA-ATCACTCATCAG-CGCTT	1497									
A.n. mt	TCCACTACATGAATAAGGAATTAATAGTAAAT-CGTGAATCA-CCATCGTCACGGTGAATTAATAA-TCAGCTGGGT-AC-ACCACTGCTCAG-CGCT	1284									
human mt	CTTAAGGGTCAAGGTTGATTTAGCAGTAAA-CTAAGAGTAG-AGTG-CTTAGTTGAACAGGGCCCTGAAGCGGCT-AC-ACACCGCCCGTCAAC-CCTCC	851									
mouse mt	CTAAGGAGT-AAGGAGGATTTAGTAGTAAA-TTAAGAAATAG-AGAG-CTTAATTGAATTGAGCAATGAAGTAG-AC-ACACCGCCCGTCAAC-CCTCC	853									
D.m. n	CGATTGAATATTTAGTGAGGTCTCCGGACGTGATCACTGTGACGCCCTTGGGTGTACGGTTGTTTCGCAAAAGTTGA---CCGAACCTTGATATTTA-G	1800									
B.m. n	CGATTGAATGATTTAGTGAGGTCTTCGGACC-GA-CACGCGGTG-CTT-CACGGC-CG-TCGGCGTTGGAAGTTGA---CCAACTTGTATTTA-G										
yeast n	CGATTGAATGGCTTAGTGAGGCTCAGGATCTGCTTA--GAGAAGGGGGCAACTCCATC-TCAGAGCGGAGAATTTGG---ACAACTTGGTCAATTTG-G	1734									
E.coli	GGGAG--TGGGTTGCAAAAGAAAT-AGGTAGC-TAAACCTTCGGGAGGGCGCTT-----ACCACTTTG-TGATTCATG	1481									
maize ch	AGGAG--CTGGCCAGGTTTGAAGTCA-TACCCTTAACCGTAAAGGAGGGGAT-----GCGTAAGGCTAGGCTTGGG	1431									
yeast mt	GAAACATATTATATCTTATTTATATAATTTTAAATAAATAAATAAATAAATTTATATTTA-----TTTATATCAGAAATAATATGA-	1590									
A.n. mt	GAAAGAAGTATGTCGAAGAAGTTTGATTTACTTATATTTATAATATATATAATCAGTTATATATATTTATAAGTTAAATTTTCGATGCATGACTTT--G	1382									
human mt	TCAAAG--TATACTTCAAAGGACATTT-AACT-AAAAACCTT-----ACGCACTTTATA	899									
mouse mt	TCAAAA--TTAATTAACCTTA-ACAT-AATTAATTTCTAGAC-----ATCCGTTTATG	902									
D.m. n	-AGGAAGTAAAGTCGTAACAAGCTTTCCGTAGGTGAACCTGGGGAAGGATCA-----TTA										
B.m. n	-AGGAAGTAAAGTCGTAACAAGCTTTCCGTAGGTGAACCTGGGGAAGGATCA-----TTA										
yeast n	-AGGAAGTAAAGTCGTAACAAGCTTTCCGTAGGTGAACCTGGGGAAGGATCA-----TTA	1790									
E.coli	-ACTGGGCTGAAGTCGTAACAAGCTTAACCGTAGCGGAACCTGGGCTGATCACTCTCTTA	1541									
maize ch	-ACTGGAGTGAAGTCGTAACAAGCTTAGCCGTACTGGAAGCTGGCGCTGATCACTCTCTTT	1491									
yeast mt	-ATTAATGCGAAGTTG-AAACAGTTACCGTAGCGGAACCTGGGCTGCTTA-----TAA	1641									
A.n. mt	-ATTGGTGTAAAGTCGAATATGCTTCGTGTAATGGAAATTGCAC-CGATGAA-----TTA	1437									
human mt	TAGAGGAGACAAAGTCGAATATGCTTAAGTGTACTGGAAGTGCACCTTGACGAAC-----	954									
mouse mt	-AGAGGAGATAAGTCGTAACAAGCTTAAGCATACTGGAAGTGTGCTTGAATAAT-----	956									

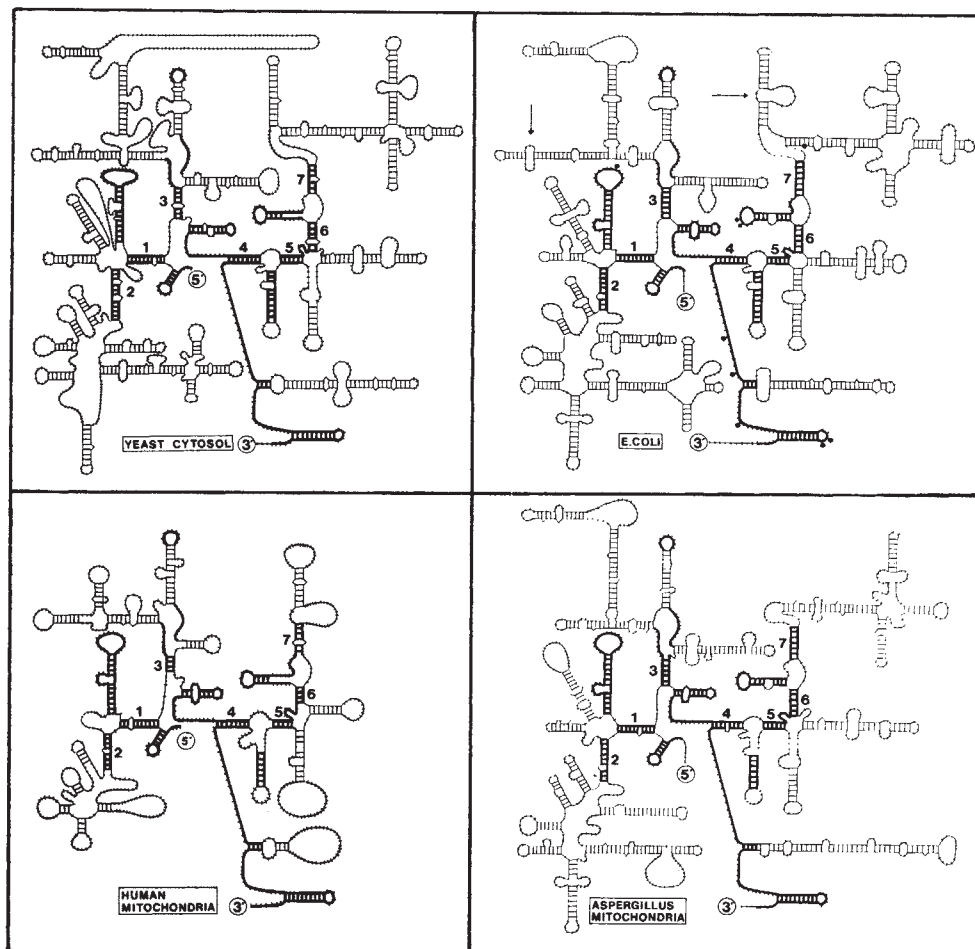


Fig. 2 Secondary structure models of S-rRNA molecules from yeast cytosol, *E. coli*, *A. nidulans* mitochondria and human mitochondria. The sequences were deduced from gene sequences (see Fig. 1 legend) and folded in analogy to the *E. coli* molecule¹⁸. The conserved structures are shown in heavy lines, and the conserved hairpin stems and far-range interactions (1-7) correspond to the boxed regions of Fig. 1. Modified nucleotides in the *E. coli* molecule are marked by dots.

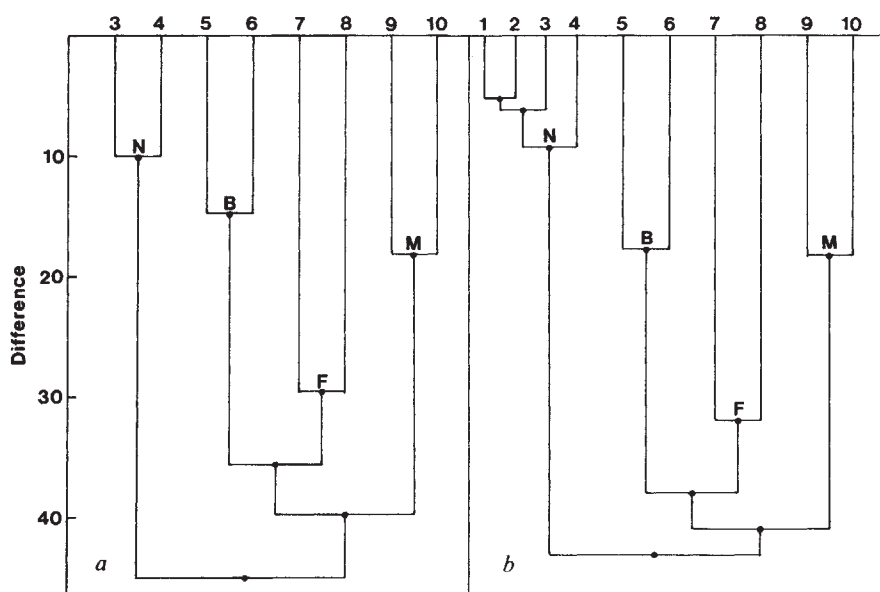
mammalian organisms, as shown by restriction analyses of primate rDNA^{22,23}. The enormous mutational rate of mammalian mitochondrial genes, manifested both as nucleotide substitutions and deletions (see Fig. 1), has certainly obscured their origins. Therefore, the relatively low preference of bacterial affinity over nuclear affinity (Table 1) is considered significant.

The sequences of regions 1 to 6 were combined to five pairs by constructing protosequences N (nuclei), B (bacteria/chloroplasts), F (fungal mitochondria) and M (mammalian mitochondria). Next, it was found that among all possible pairs

of protosequences the pair [B,F] had the lowest substitutional distance. The analysis finally resulted in the tree topology [(B,F)M]N as shown in Fig. 3a.

The alternative topologies [(F,M)B]N (common mitochondrial root, prokaryotic affinity), [(F,M)N]B (common mitochondrial root, eukaryotic affinity) and [(F,B)(M,N)] (prokaryotic affinity of fungal, eukaryotic affinity of mammalian mitochondria) were also tested, but were found to be much less favourable, because the order of successively determined nodal points (differences between protosequences) was reversed in all three cases. The same analysis was performed with the 3'-

Fig. 3 Evolutionary trees of S-rRNA gene sequences, based on six non-deleted regions (a) and two conserved 3'-terminal regions (b) of the sequence alignment. The tree topology was determined by the matrix method of Fitch and Margoliash²⁰ (see difference matrix of Table 1). Protosequences were constructed as previously described²¹. To each nucleotide or gap of an original sequence the index 1.0 was given. For all positions of a given pair of sequences (which may consist of two original sequences, two protosequences or a combination of both), the 'nucleotide composition' (reflecting the probability that a given nucleotide was at this position in the ancestral sequence) was determined by adding up the one-half indices for each nucleotide at a given position. The difference between two (proto)sequences was determined as follows. For each position the product of indices for all possible pairs of different nucleotides was determined (gap versus nucleotide: one-half product), and the sum of all products for a given pair of sequences (= difference, expressed as per cent of all compared positions) was plotted as nodal point in the tree diagram. Sequences: nuclear genes of *D. melanogaster* (1), *B. mori* (2), *X. laevis* (3), *S. cerevisiae* (4) (N), *E. coli* (5), maize chloroplasts (6) (B), mitochondrial genes of *A. nidulans* (7), *S. cerevisiae* (8) (F), human cells (9) and mouse (10) (M). Numbers in parentheses refer to branch numbers (top of dendrogram).



terminal regions 5+6 (including two nuclear sequences from insects, Fig. 3b). The tree data clearly support the idea¹ that mitochondria have originated from endosymbiotic bacteria²⁻⁴. The alternative hypothesis, an origin of mitochondria genes by duplication, excision and intracellular compartmentalization of chromosomal genes^{5,6}, would predict a common root between mitochondrial and nuclear genes which is not apparent from our data.

The tree topology of Fig. 3 suggests an independent origin of fungal and mammalian mitochondria, because a common root could not be found for both groups of sequences. This interesting possibility can be further tested by determining and comparing S-rRNA gene sequences from early diverging eubacteria like *Thermus aquaticus* and *Rhodospirillum rubrum* which are possibly more related to bacterial precursors of mitochondria than *E. coli*^{3,21}. The idea of an independent invasion of different bacteria into already diverged proto-karyotic precursors to animal and fungal cells is attractive, because the mitochondrial genomes of the two kingdoms differ in several fundamental properties like gene organization, tRNA structure, genetic code and codon usage^{24,25}.

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An archaeobacterial 5S rRNA contains a long insertion sequence

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Sequence characterization of 16S rRNA has revealed an ancient divergence that divides prokaryotic organisms into eubacteria and archaeobacteria¹. This evolutionary split is so fundamental that by the 16S rRNA criterion, the archaeobacteria appear to be as distinct from eubacteria as they are from eukaryotes². Extensive comparison of the lipids³ and cell walls^{4,5} of the various archaeobacterial species strongly supports this interpretation. Detailed molecular investigations of the archaeobacteria have provided surprising findings concerning the RNA polymerases^{6,7}, the translation system^{8,9}, the rRNAs¹⁰ and the 5S rRNA^{11,12}. Here we report the occurrence of a 231-nucleotide archaeobacterial 5S rRNA homologue containing a 108-nucleotide segment in its interior that is not homologous to any region of a closely related normal-size 5S rRNA.

To investigate further the evolutionary relationships among the archaeobacteria we have undertaken a comprehensive comparison of the 5S rRNAs of numerous archaeobacterial species. Especially noteworthy was the apparent absence of a 5S RNA molecule in phenol-extracted cell lysates of *Halococcus morrhuae* ATCC 17082 and the occurrence of a novel band corresponding to a larger RNA. Subsequent examination of purified ribosomal preparations confirmed the absence of normal-size 5S rRNA and established that the new larger RNA was, in fact, a ribosomal component. The latter was shown (see Fig. 1) to have the following sequence.

```
pUUAAGGCGGCCACAGCGGGCGGCGGACUCCCGUACCCCAUCCCGAACACGGCAGAU
AAGCCCGCCAGCGGUUCCAGCGAGUACUGGAGUGUGCGGAACCCUCUGGGAAAAACUGGC
UCGCAAGAGGGGCCAAGUGUGAGCCAGGAGCGCAUCCGUGUACACACGGCGAGUC
CGUCCCGGUGGAACCCGAGACCGCUCGCGUUAACAACGGCUCAUAACUAGUGGUUCCG
CGCCUCCCOH
```

The two underlined segments were particularly difficult to determine due to severe band compression. The interpretation in both these regions relies on gels reading from one direction only with collaborating oligonucleotide catalogue data. Thus, the sequences of these areas are less definite than the rest of the sequence. Band compression also made it impossible to verify fully the cytosine at position 19.

Comparison of the first 108 nucleotides with the known *Halobacterium cutirubrum* 5S rRNA sequence (Fig. 2) reveals 89% sequence homology without recourse to any insertion or deletion events and establishes that this novel rRNA is in fact a 5S rRNA homologue. Beginning at position 108, the homology abruptly disappears and does not recur until 108 nucleotides later where positions 216-231 of the large RNA are 87% homologous with positions 109-123 of *H. cutirubrum*. Apparently a mutational event(s) has resulted in a large insertion in the interior of the 5S rRNA without interfering with its ability to participate in protein synthesis. Despite the numerology, the primary sequence data do not suggest a duplication event.

The only other reported example of a significantly larger 5S rRNA is in *Clostridium thermosaccharolyticum*¹³ where the cells contain two 5S rRNAs, one of normal size and another with an additional 40 nucleotides appended to the 3' terminus (ref. 13 and K. R. Luehrsen and C. R. Woese, unpublished data). It is not known whether the larger variant is functional. In view of these facts the two phenomena seem unlikely to be related.

Of interest is the manner in which the *H. morrhuae* 5S rRNA homologue is adapted to the structural requirements of the ribosomal machinery. Although the details of the 5S RNA secondary structure are not yet certain, comparative sequence analysis has shown that a minimal model containing five helical regions is applicable to eukaryotic cytoplasmic 5S rRNA¹⁴. The structure of eubacterial 5S rRNA, although similar, is distinct in that it only contains four helical regions¹⁵ of three or more contiguous Watson-Crick pairs. Recently several extensions of these helical regions have been found to be consistent with the comparative data when certain looped-out nucleotides are allowed¹⁶⁻¹⁸.

Archaeobacterial 5S rRNAs from *H. cutirubrum*¹¹ and *Thermoplasma acidophilum*¹² do not conform exactly to either of these models, but have features of both. In the case of *H. cutirubrum*, a secondary structure has been proposed¹¹ that, when modified to include the appropriate extensions to the helical regions, closely resembles eukaryotic cytoplasmic 5S rRNA. The most notable differences are: (1) helix V is shorter than usual so that it may not be possible to form coaxial helices between helices I and V or between helices II and V. This is in contrast to eukaryotic cytoplasmic 5S rRNAs and *T. acidophilum* 5S rRNA where such alternative coaxial helices are obviously possible¹⁴. (2) The loop defined by helix III is characteristically eubacterial in that it contains 13 nucleotides and the sequence segment CGAAC. Most relevant here is that the homologous regions of the *H. morrhuae* sequence (Fig. 2)

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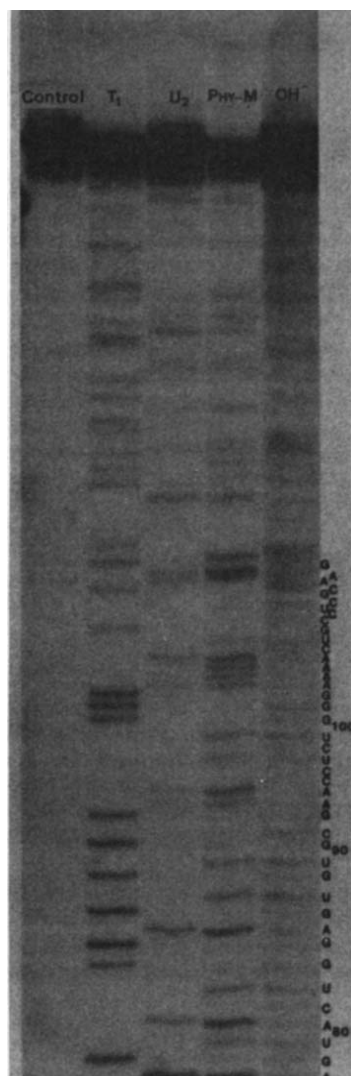


Fig. 1 Sequence of *H. morrhuae* 5S RNA homologue. *H. morrhuae* ATCC 17082 was grown in van Neil's yeast medium supplemented with 25% NaCl (ATCC medium 217). Cells were collected in stationary phase by low-speed centrifugation (8,000g for 15 min) and ground with twice their net weight of alumina (type 305). The paste was resuspended in a buffer volume equal to three times the wet cell weight²⁰ (10 mM Tris-HCl pH 8.0, 50 mM KCl, 100 mM Mg acetate, 6 mM 2-mercaptoethanol), and the alumina and large cell debris removed by centrifugation (8,000g for 20 min). The supernatant was further clarified at 30,000g for 1 h and then layered over a pad of 1.5 M sucrose dissolved in the above buffer and spun for 11 h at 100,000g. DNA was carefully removed from the top of the pellet which was then resuspended in 0.3 M Na acetate and dialysed against the same salt solution. The dialysate was extracted with 2 vol of saturated phenol and the aqueous layer precipitated with 3 vol of EtOH. The various rRNA species were separated and purified by polyacrylamide gel electrophoresis (10% acrylamide, 7 M urea, 50 mM Tris, 50 mM boric acid, 1 mM EDTA) at 20 V cm⁻¹ for 3 h. Both *in vitro* ³²P 5'- and 3'-end-labelled^{21,22} RNAs were used in sequence determination. The terminal regions were characterized as described elsewhere¹⁴. To sequence the interior regions, both 5'- and 3'-labelled 5S and 5'-labelled fragments obtained by partial digestion with RNase T₂ (ref. 23) were analysed by rapid gel sequencing methods. The enzymatic method²² was used extensively with the cytosine/uracil distinction enhanced by the use of the recently described *Physarum*-M (Phy-M) nuclease²⁴. The chemical method²⁵ was also used on 3'-end-labelled RNA. Sequencing ladders were run on thin (0.02 cm) 20% polyacrylamide gels at 2.0 kV. Pancreatic and ribonuclease T₁ oligoribonucleotide catalogues were determined¹ and provided an independent check on the sequence results reported here. The gel shown was obtained by the enzymatic method on a fragment produced by prior digestion of whole 5S rRNA with ribonuclease T₂.

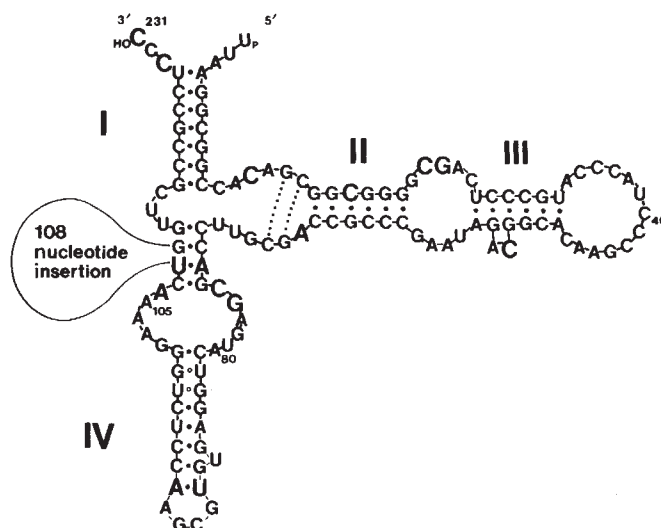


Fig. 2 The *H. morrhuae* sequence is shown in a modified version of the secondary structural arrangement proposed for *H. cutirubrum*¹¹. The insertion is shown as a looped-out region beginning and ending in what would usually be helical region V. The sequence of the insertion is given in the text. The sites of the 15 sequence differences relative to the revised (K.R.L. and G.E.F., unpublished data) *H. cutirubrum* sequences are indicated by the larger letters. In detail, according to a numbering system based on the *H. cutirubrum* sequence the changes from *H. cutirubrum* to *H. morrhuae* are (no.) 13 U → A, 19 U → C, 24 U → C, 25 U → G, 40 C → A, 51 A → C, 65 U → A, 73 G → A, 76 C → G, 89 C → U, 94 G → A, 106 U → A, 108 C → U, 121 A → C and 123 C → U. These include a coordinated pair, nos 89 and 94, that is consistent with the extension of helix IV proposed for eukaryotic 5S rRNAs¹⁶.

conform exactly to the likely *H. cutirubrum* structure with the single exception that formation of the helical region V is thermodynamically suspect because it requires the looping out of the entire 108-nucleotide insertion. It is therefore unlikely that the insertion has seriously disrupted the usual structure of the homologous regions. This is further indicated by our observation that the characteristic¹⁹ nuclease sensitivity in the loop defined by helix III is present in *H. morrhuae* 5S rRNA. A reasonable interpretation is that the nucleotides of the 108-base insert fold into a separate structural domain that can be spatially accommodated in the ribosome. An automated search for possible helical regions in the insert did reveal some prospects, particularly the pairing of positions 128–135 with 201–209 in conjunction with either 138–143 with 146–150 and 171–175 with 188–194 or 159–164 with 189–195.

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Nature of the charge distribution in proteins

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The significance of the fairly strong electric fields produced by the electric macrodipole of the α -helix and by ionic charges in stabilizing globular proteins has been recognized for some years¹⁻⁴. These electrostatic interactions are involved at the active sites of functional proteins in binding a substrate or a coenzyme, or in enhancing enzymatic reaction rates²⁻⁵, and are unique among the many interactions affecting protein structure and function because of their long range, extending over the whole protein molecule. We have now analysed the distribution of the distances separating the ionic charges (ionized groups and the apparent charges at the termini of the α -helices) for more than 44,000 charge pairs in 14 proteins. Our results show that charges in the proteins are, on average, surrounded by charges of opposite sign. Previous calculation of the electric potential near the helix termini has shown that the electrostatic effect of the α -helix dipole is equivalent to the effect of one-half of a positive unit charge at the N-terminus of the α -helix and one-half of a negative unit charge at the C-terminus³. We report here that the macrodipole of the α -helix has the same order of contribution to stabilizing the native protein conformation as ionized groups.

We have calculated the parameter $\alpha_{ij} = e_i e_j / r_{ij}$, where e_i and e_j are the charges of the i th and j th residues of a protein and r_{ij} the distance between them. This calculation has been done for all the interactions in 14 proteins analysed, and we have presented the results in graphic form where $\Sigma \alpha_{ij}$ is plotted against r_{ij} (with steps of 2 Å). Although the parameter is a form of Coulomb interaction having a dimension of energy, it is used as a geometrical measure to evaluate the charge distribution. Electrostatic energy calculations, taking into account the internal dielectric constant of protein and the effect of counter ions, will be done below. The attractive ($\Sigma_{+-} \alpha_{ij}$) and repulsive ($\Sigma_{++--} \alpha_{ij}$) interactions have been analysed separately, as shown in Fig. 1. Figure 2 shows a plot of the difference (Δ) between these results versus r_{ij} . Figure 3 only plots interactions involving helix termini (that is, charge-charge interactions are excluded but not charge-helix dipole interactions). The graphs show how the same and opposite charge distributions vary with the distance between the interacting groups in the proteins studied.

The distances of the charge separations, r_{ij} , are obtained from the crystallographic structural data at Å resolution in the protein data bank, a computer-based archive for macromolecular structure. The locations of the charges at side-chain residues and helix termini are assigned as follows: for $-\text{COO}^-$, one negative electronic charge ($-|e|$) is located at the centre of a line which joins the two oxygens. One negative and one positive charge ($-|e|$ and $+|e|$) are located at the sulphur atom and the nitrogen atom for $-\text{S}^-$ and N^+ residues respectively. A positive (or negative) charge of $0.48 \times |e|$ is located at the centre of the $\text{C}^\alpha\text{--N}$ bond of the amino acid at the positive (or negative) terminus of the helix³. A few side chains (<0.1% of the total) whose atomic coordinates have not been established are not included in the present calculation. The degrees of ionization of the charges, e_i , are calculated according to the Tanford-Kirkwood equation⁶, taking into account their intrinsic pK values and the effect on them of the electrostatic interactions with surrounding charges. Recursion calculations of at most 20 times gave the final charge value for each ionizable group. The internal

dielectric constant is assumed to be 4 and the ionic strength is 0.1, pH 7.

The difference-distribution profile in Fig. 2 exhibits a distinct minimum at a small inter-charge distance. This kind of profile is approximately typical of each protein examined. For each protein, the distances at which the curve shows a minimum (that is, maximum negative value), $r_{\min}$, range from 2 to 10 Å; the mean value $\bar{r}_{\min} = 5$ Å and the mean deviation $\langle \Delta r_{\min} \rangle = 1.5$ Å. The situation is the same in Fig. 3. Thus a charge in the protein, including apparent charges of the helix dipole, is surrounded, on average, by charges of opposite sign.

In an ionic solution, according to the Debye-Hückel picture, the immediate neighbourhood of an ion is more likely to include an ion of opposite electric charge than one of the same sign. This is a dynamic compromise between electrostatic interaction and thermal agitation. A similar situation seems to result in the charge distribution in proteins. The residues in proteins, however, have a regular conformation around a fairly rigid backbone structure, whose freedom of motion is highly restricted. Although residues in globular proteins clearly have a considerable degree of internal freedom of motion, internal flexibility alone is not sufficient to produce a positional rearrangement of charges as is found in liquid phases. Therefore, the charge distribution in the protein, compared with that in an ionic solution is not entirely the result of the dynamic equilibrium; it must be the equilibrium attained during the long evolution of the protein.

The pressure selecting the charge distribution typical of present-day proteins, whether it has functioned during the course of protein evolution or is a dynamic equilibrium, may simply be energetic. Quantitative evaluation of the electro-

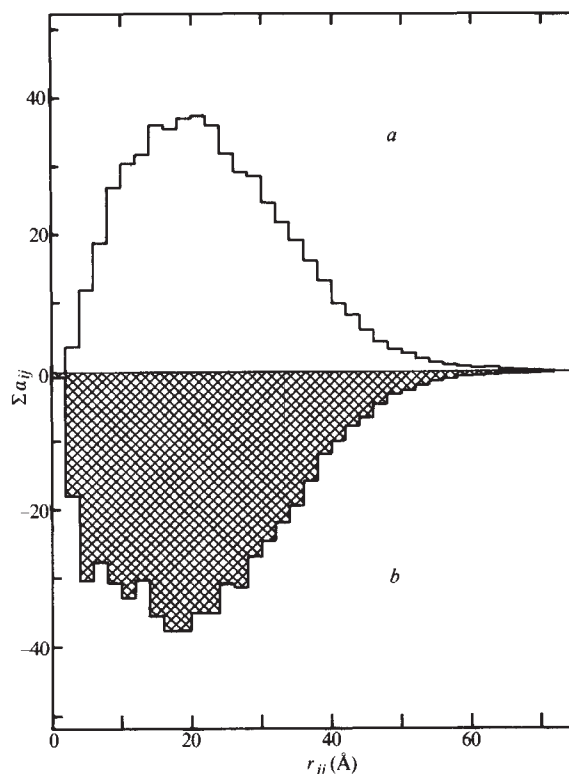


Fig. 1 Histograms showing the distribution of distance, r_{ij} , between 44,592 pairs of charges (on residues i and j) in 14 proteins. Values are given for 2 Å increments. *a*, In the positive region, the plot is for pairs of charges of the same charge (repulsive interactions); *b*, in the negative region, interactions are between opposite-sign pairs (attractive). All pairs of ionic-ionic, ionic-dipolar and dipolar-dipolar charges are taken into consideration. Dipolar charge denotes the half unit charge ($0.48 \times |e|$) that is allocated at the end of the α -helix. The source proteins are listed in Table 1.

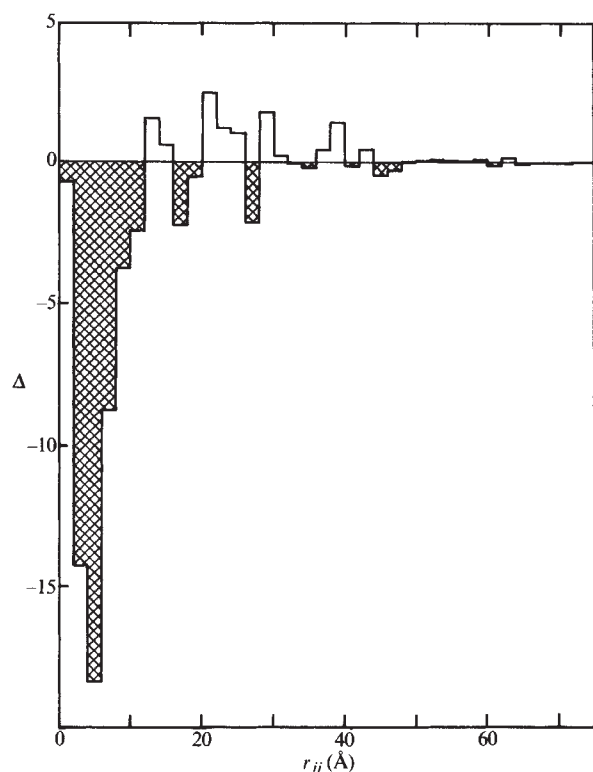


Fig. 2 Plot of the difference (Δ) between the two curves shown in Fig. 1.

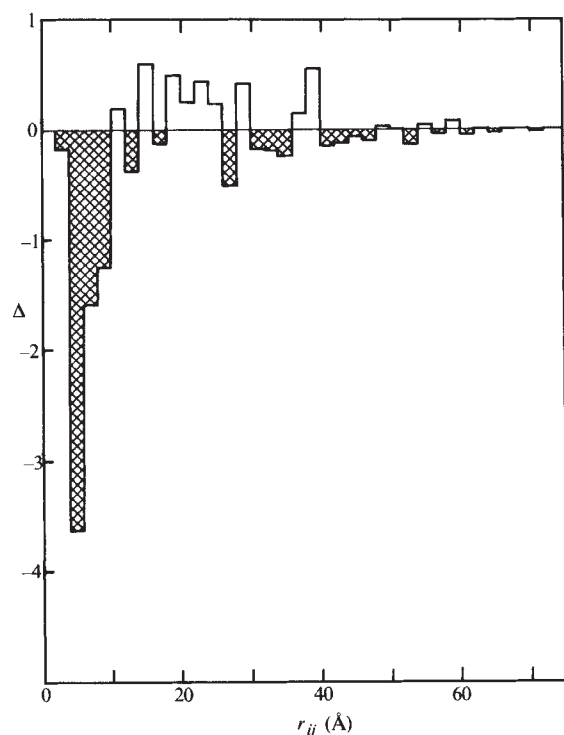


Fig. 3 Difference plot showing the distribution of separation between the dipolar charge and all ionic and dipolar charges (ion-pair interactions are ignored in this plot). 13,258 pairs from 14 proteins are included.

static energy which may tend to produce the Debye-Hückel-type charge distribution in proteins encounters two problems: the ambiguity of the value of the internal dielectric constant in protein and the estimation of the contact surface of the protein with water. Although the internal dielectric constant has been estimated⁷ to be in a range of 1–60, here we adopted the value 4 as an averaged value because it is that most often used in

Table 1 The energy of charge-charge interaction in 14 proteins

	U_{el} per protein (kcal mol ⁻¹)	U_{el} per charged residue (kcal mol ⁻¹)	U'_{el} per helix end (kcal mol ⁻¹)
Myoglobin (deox.) (sperm whale)	-454	-13.7	-4.4
Concanavalin A (jack bean)	-168	-5.6	—
Lysozyme (hen egg-white)	-71	-3.6	-2.8
Papain (papaya latex)	-126	-3.7	-3.6
Ribonuclease A (bovine pancreas)	-75	-3.0	-7.1
Trypsin inhibitor (bovine pancreas)	-7	-0.3	-3.8
Adenylate kinase (porcine muscle)	-362	-8.8	-4.6
Carboxypeptidase A (bovine pancreas)	-338	-8.3	-1.4
Glyceraldehyde-3-phosphate dehydrogenase (lobster)	-563	-11.4	-2.4
Alcohol dehydrogenase (horse liver)	-595	-10.8	-2.3
Lactate dehydrogenase (dogfish muscle)	-452	-7.9	-4.4
Cytochrome C (ox.) (tuna heart)	-71	-2.6	-4.4
Carbonic anhydrase B (human)	-326	-8.3	-4.7
β -Trypsin (pH 8) (bovine pancreas)	5	0.6	-1.9

U_{el} is the total electrostatic energy. U'_{el} is the energy of the interaction between ionic and dipolar charges. The internal dielectric constant of the protein, ϵ , is assumed to be 4.

similar calculations. The Tanford-Kirkwood algorithm contains the internal dielectric constant, so that, in principle, α_{ij} is affected by it. However, at neutral pH, the condition of the present calculation, the geometric parameter α_{ij} changes little with a change in the internal dielectric constant. A spherical contact-surface was simply assumed, and its radius was calculated from the molecular weight and the partial specific volume. The resulting electrostatic energies of the proteins are listed in Table 1.

The ionic charge is found to obtain an electrostatic stabilizing energy of several kcal per ionic residue, and the apparent charge of the α -helix dipole dose also has the same order of energy per helix end. Thus the present statistical examination shows that, on average, the electrostatic interaction among the ionic and the dipole constituents exceeds the energy of thermal agitation, and, therefore, that they are located so as to stabilize the protein structure.

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Erratum

THE words on the cover of *Nature* of 10 September 1981 (issue no. 5828) were incorrect and should have read 'Newly generated nuclei in adult rods'. The cover photograph (relating to the letter 'Genesis of rods in teleost fish retina' by P. R. Johns and R. D. Fernald *Nature* **293**, 141–142) was taken by R. D. Fernald and E. Newman of the Institute of Neuroscience, University of Oregon.

MATTERS ARISING

Stability of zeolites under electron irradiation

RECENT electron microscopic studies^{1,2} of structural changes in zeolites under electron irradiation have led to the plausible assumption that water, normally contained in zeolites, is linked with the process. It is thought² that the high-intensity electron bombardment, used for high-resolution electron microscopy, produces significant numbers of OH⁻ ions by electron impact. These ions are in turn believed to weaken the bonds of the aluminosilicate framework, causing its eventual collapse into a disordered state.

It seems likely that the effect observed by Bursill *et al.* is related to a type of degradation through electron, ion or photon irradiation, that I previously reported, which appears to occur in all substances containing M⁺ cations of the alkali metals or alkaline earths and which is therefore expected to take place also in zeolites. My colleagues and I believe³ that the primary function of the irradiation is not to produce OH⁻ radicals, but both to detach cations from their normal positions according to



and to form catalytically active surface centres where they are converted from M⁺ to M.

In the presence of H₂O



and



At suitable surface sites, the OH⁻ produced in step (3) can break the M-X bond so that



following which steps (3) and (4) are cyclically repeated as long as water vapour is available.

Note that H₂O need not be present during the actual irradiation, but can be admitted later, and once the catalytic centres are formed and reaction step (1) completed, the irradiation may be discontinued, as in each reaction cycle the OH⁻ radical necessary for its successor is produced.

This was in fact the experimental situation in our work, but in the investigations of Bursill *et al.*, H₂O was available throughout, and as their bombardment intensity exceeded ours by several orders of magnitude, impact ionization could have provided a second, and perhaps even dominant, source of OH⁻, thus explaining the very fast reaction rate implied by their observation of significant breakdown within only a few minutes.

In contrast to this, although we have not experimented specifically with zeolites, our experience with other substances suggests that in the absence of significant impact ionization, such as when dehydrated materials are exposed to a moist atmosphere after irradiation, some weeks or even months may have to elapse before degradation becomes evident. Experiments along such lines might assist in elucidating the reported instability of zeolites.

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BURSILL ET AL. REPLY—We thank Hirsch for his remarks. In ref. 1, he has proposed a reasonable mechanism for the production of OH⁻ in argon ion-bombarded bulk specimens in the presence of moisture.

In their normal condition, zeolites are very rich in water content—up to ~50% by volume in some cases—so that the passage of 200 KeV electrons through

such materials is similar to the production of an electrical discharge through water. Under these circumstances, it would not be surprising for the rate of production of OH⁻ to be considerably higher and for the rate of destruction of the crystal to be commensurately greater than in the situations cited by Hirsch. One fact stands out: thorough dehydration of zeolites before their examination by electron microscopy is crucial in enhancing the beam stability so as to permit high-resolution imaging of the kind we described²⁻⁵. The mechanism proposed by Hirsch may be more relevant to dehydrated than hydrated zeolites.

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Mechanisms of slow postsynaptic potentials

THE review article by Hartzell¹ contains factual errors with regard to neuro-glandular synapses. His Table 1 lists information on cat salivary glands and pancreas. The data given in respect of cat pancreatic acinar cells are wrongly cited, misleading and partly non-existent.

To my knowledge, microelectrode measurements on cat pancreas have only been reported once², where it was shown that exogenous acetylcholine (ACh) caused membrane depolarization (not hyperpolarization as claimed in the review article¹), but no information was given on latencies, duration of responses or ionic mechanism. Acinar membrane potential changes following nerve stimulation were not described for the cat pancreas.

All this information is, however, available for mouse (and rat) pancreatic acinar cells (see Table 1 for values and refs). I have also listed information for mouse parotid acinar cells because ionic mechanisms have been investigated in much greater detail in this species than in the cat. Substance P and adrenaline applied to rat parotid acinar cells by micro-ionophoresis evoke membrane potential changes that have a reversal potential and latencies also characteristic of the action of ACh³. Peptides belonging to the cholecystokinin and bombesin groups act on pancreatic acinar cells by evoking potential and conductance changes similar to those evoked by ACh⁴, although these peptides interact with two types of receptor site distinct from the muscarinic receptors⁵. The minimum latencies found for peptidergic cell activation were 500–1,500 ms (ref. 4).

Table 1 Selected examples of slow postsynaptic potentials in mouse gland cells

Tissue	Response	Δ Conductance	Latency (ms)	Duration (s)	Transmitter	Refs
Parotid acinar cells	Biphasic: depol.; hyperpol.	↑Na ↑K	250	2–5	ACh	7, 8
Pancreatic acinar cells	Depol.	↑Na ↑Cl ↑K	900	5–20	ACh	9, 10

Neuroglandular transmission involving these peptides has not been demonstrated electrophysiologically. The long latencies for neurotransmitter and peptide hormone action on pancreatic acinar cells are in marked contrast to the short-latency membrane depolarization and conductance increase evoked in the same cells by some neutral amino acids⁶.

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Transcurrent faulting and pre-Carboniferous Anglesey

NUTT AND SMITH¹ invoke Devonian transcurrent faulting to explain differences of pre-Carboniferous geology between Anglesey and mainland North Wales. They postulate large dextral movement from proposed geological compatibility between the Lake District and North Wales. We suggest that such movement is unlikely along the only on-land occurrences of the postulated fault in Llyn and Cumbria.

Current sedimentological work by one of us (G.D.T.) in south-west Llyn indicates close similarities in the Arenig sequences across the proposed fault. On both sides, thin granular-pebbly sandstones (dominantly Monian derived) immediately overlie the basal Arenig unconformity, and upper *Didymograptus extensus* zone sedimentation changes from sand to mud-silt dominated, which persists throughout the Llanvirn. Such differences as exist are readily interpretable shallow marine facies variations. The suggested Llyn faultline would truncate continuous Arenig-Llanvirn outcrop, and no alternative seems stratigraphically and structurally acceptable.

In Cumbria, the postulated fault follows the Dent line. Its visible, post-lower Carboniferous displacement is dip-slip only. Post-Downtonian strike-slip faults do parallel this line to the west, but have dominantly sinistral displacement. Significantly, pre-Devonian features appear continuous across the Dent Line. Thus the arcuate Lake District structure continues into the Craven Inliers, and the sedimentary-volcanic Ashgill sequences of the Cautley and Craven Inliers match closely. A more suitable basement fault line is beneath the E-W Craven Fault Belt and perhaps eastwards beneath the Coxwold-Gilling and Helmsley-Filey Fault Belts.

Lake District and North Wales compatibility is equivocal. Their Ordovician correlation is no stronger than would be expected within the same plate tectonic environment. For example, Ashgill lithofacies similarities do not outweigh their

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imprecise biostratigraphical correlation. The absence of Rawtheyan silicic volcanicity in North Wales is also important. Furthermore, structural arcuation in North Wales has a NW-SE axis whereas that in the Lake District is more N-S. We conclude that, if Nutt and Smith's model is to be viable, some rotation must be invoked, with a revised on-land fault line.

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NUTT AND SMITH REPLY—In the Llyn, the proposed fault between Porth Nefyn and the western side of Hell's Mouth runs to the west of Dinas and curves southwards to the east of Sarn. This line, roughly parallel to the strike^{1,2} and sediment drift³ of the Ordovician rocks, is largely covered by superficial deposits, as Matley's field maps (in the possession of the Institute of Geological Sciences) show. The line does not appear to cross either continuous stratigraphical or structural features. In the southern part of the area, where Matley postulates² a fault, the scattered solid outcrops show evidence of contorted strata. We have not had access to Tegerdine's current sedimentological work, but published information indicates substantial differences, commented upon by Nicholas⁴, between the Ordovician sequence to the west of the above line^{1,5} and those to the east at St Tudwal's⁴ and in the area south of Carn Fadryn². In particular, the western sequence contains both acid and basic volcanics with associated intrusives, all of which are unrepresented in strata of equivalent age to the east. According to Crimes³ the Arenig arenaceous rocks of the Llyn are essentially derived from the south-west, especially on the eastern side of the proposed fault, and a Monian origin for the sandstones therefore supports rather than

detracts from the transcurrent fault hypothesis.

Post-Lower Carboniferous movement along the Dent Line is irrelevant to the argument, but Moseley⁶ has shown that many of the Variscan dip-slip faults in the Lake District have a history of strike-slip movement in the Caledonian. Sinistral wrenches in the area are not parallel to the proposed fault, but are northerly trending⁶ and probably represent conjugate fractures. We cannot agree that pre-Devonian structures⁶ cross the Dent Line. Nor do the Cautley and Craven sequences match as closely as Tegerdine *et al.* suggest; thus the acid tuffs of the Ashgill in the former area contrast with thin beds of tuff and tuffite in the latter and the Borrowdale Volcanics of Cautley are absent in the Craven Inlier. All other factors apart, basement magnetic anomalies⁷ show that the Dent Line is to be preferred to a Coxwold-Gilling-Filey line for the proposed transcurrent fault.

We cannot agree that there is incompatibility between the structural arcuation of North Wales and the Lake District before the proposed translation. Subsequent rotation is apparent (see our Fig. 1) because the fault line is not straight.

1. Matley, C. A. *Q. Jl geol. Soc. Lond.* **88**, 238-273 (1932).
2. Matley, C. A. *Q. Jl geol. Soc. Lond.* **94**, 555-606 (1938).
3. Crimes, T. P. *Proc. geol. Ass.* **81**, 221-240 (1970).
4. Nicholas, T. C. *Q. Jl geol. Soc. Lond.* **71**, 83-143 (1915).
5. Matley, C. A. *Q. Jl geol. Soc. Lond.* **84**, 440-504 (1928).
6. Moseley, F. *J. geol. Soc. Lond.* **128**, 561-598 (1972).
7. Institute of Geological Sciences 1:625,000 Aeromagnetic Map of Great Britain, Sheet 2 (1965).

THE oldest rocks on Anglesey form the Mona Complex¹ which is thought to be the remnant of a subduction complex² once active on the south-east flank of the Iapetus Ocean during the earliest Cambrian^{3,4}. Dating evidence within the complex is poor, but suggests that the complex is no younger than Lower Cambrian³. The oldest rocks exposed on the south-east side of the postulated strike-slip fault⁵ are the Arvonian acid tuffs which are succeeded conformably by Lower Cambrian sediments⁶ which contain fragments derived from the Mona Complex^{1,7}. Some of these fragments are schistose (the highly distinctive Penmynydd Schists)¹ and indicate that deformed and metamorphosed Monian rocks were being eroded and incorporated as clastic debris in the Welsh Basin sediments during the Lower Cambrian. As the Arvonian tuffs show none of the structural and metamorphic complexity typical of the Mona Complex, and as these tuffs grade up in places into the overlying conglomerates of the basal Fachwen Formation⁸, it is reasonable to deduce the tuffs to be post-Monian. Thus the Monian subduction system has probably been active in the earliest Cambrian but was certainly uplifted and exposed before the

eruption of the Arvonian tuffs and the subsequent deposition of the Cambrian Welsh Basin sediments.

Nutt and Smith give the Arvonian a Precambrian age, although there is little evidence for this. Monian and Arvonian are believed to be Precambrian¹ because they are older than the late Lower Cambrian sediments of the Welsh Basin. The discovery of Palaeozoic acritarchs in the Monian (Gwna Melange) places some of the Mona Complex in the Cambrian but does not alter the field relationships indicating a post-Monian age for the Arvonian; the latter should therefore be considered as more likely to be of Cambrian rather than Precambrian age.

The correlation of the Baron Hill Beds and Bwlch Gwyn Felsite on Anglesey with the Arvonian on the mainland^{1,2} is dismissed by Nutt and Smith as "an unlikely correlation". They do not explain why it is unlikely and do not offer a reason for disagreement or an alternative correlation. Greenly's correlation is certainly inconvenient to the strike-slip hypothesis. These exposures of so-called Arvonian in Anglesey need to be restudied in detail.

If Nutt and Smith can prove that "there is stratigraphical evidence (unpublished) showing that at least part of the Gwna *mélange* is of Ordovician age" then it would indicate that the development of the Monian subduction complex was coeval with that of the Welsh Basin. Such evidence could transform our understanding of North Wales geology. However, until conclusive evidence for an Ordovician Gwna *mélange* is presented it would seem wiser to accept the published evidence which indicates an age no younger than Lower Cambrian for the *mélange*⁴.

On Llyn, the postulated major strike-slip fault is suggested to run from the west side of Hell's Mouth north-east to Porth Nefyn. Both these coastal localities are covered by thick glacial drift. Inland exposure is poor and there is no field evidence published in support of the existence of such a major fault⁷ (G. D. Tegerdine, personal communication). Yet Nutt and Smith state that this postulated fault separates "substantially different Ordovician sequences", but offer no explanation. Furthermore, the postulated fault appears to cut across one of the end-Silurian to Devonian metamorphic isograds (pumpellyite) in the Ordovician on Llyn recently mapped by Roberts¹³. There seems to be a distinct lack of evidence in support of a massive 100 km separation between the Ordovician on either side of a line drawn north-east from Hell's Mouth. Such evidence is, however, vital to Nutt and Smith's argument because the Arenig on western Llyn (west of the postulated fault) rests with clear unconformity on a Monian basement¹⁴⁻¹⁶.

Greenly^{1,7} identified Monian pebbles in all three main outcrops of Cambrian rocks in the north Welsh basin (north-west

Gwynydd, St Tudwal's Peninsula, Harlech Dome). It is difficult to see how these fragments were derived if the Mona Complex lay 100 km further south-west. According to Nutt and Smith the Mona Complex would have lain nearer to Pembrokeshire, yet Monian-type fragments are not a characteristic of the Cambrian sediments there^{17,18}.

The existence of the proposed major dextral transcurrent fault is not supported by the regional mid- to late-Devonian tectonic regime. The concept of major dextral transcurrent along the NE-SW faults seems to have originated from the oblique collision model¹⁹ which supposedly resulted in up to 1,000 km of dextral strike-slip along the subducted Iapetus suture. However, the absence of any transcurrent movements within the large area of early Devonian Cheviot lavas lying astride the suture constrains any movements to a Pre-Devonian age²⁰. It seems safer to return to the earlier arguments favouring a sinistral transcurrent for such faults as the Great Glen²¹ and equivalent Leannan²² and Leck²³ Faults in Eire. It is difficult to reconcile large dextral movements along similarly oriented faults of similar age, such as those suggested by Nutt and Smith, without invoking the unlikely idea of some form of major stress reversal.

Most of the contrasts between Anglesey and the mainland are easily explicable in other ways, particularly if the Mona Complex is older than the Welsh basin. Even if new evidence arose to prove the two were coeval this would still not necessitate a major separation between them because the close juxtaposition of rocks with dramatically different geological histories is quite normal within subduction systems.

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NUTT AND SMITH REPLY—The supposed occurrence of Monian fragments in the Lower Cambrian of the Welsh mainland derives from Greenly's observations¹. We place little reliance on Greenly's pebble identifications in this instance, subsequent workers²⁻⁵ having gone no further than to say that derivation of the pebbles was from a metamorphic landmass similar to Anglesey, but with differences. No pebbles of rocks peculiar to Anglesey have been found in the Lower Cambrian, Greenly¹ having observed the absence of glaucophane schists, and it is these that are distinctive of the Penmynydd Zone. Thus the schistose fragments referred to by Gibbons and Gayer could have come from anywhere.

There is no absolute evidence to show whether the Arvonian (of the mainland) is Precambrian or Cambrian. It is currently⁶, and traditionally¹, considered to be Precambrian. The "field relationships indicating a post-Monian age for the Arvonian" amount to circumstantial evidence consisting of pebble recognition (see above) and an assumed correlation between the Arvonian and the Anglesey 'Arvonian' (see below). The assumption that metamorphosed and structurally complex rocks are older than rocks that are less altered and disturbed is dangerous in a subducting-plate environment. Thus large parts of the Mona sedimentary sequence may be the age equivalents of Cambrian rocks on the mainland.

There is no positive evidence for the age of the Bwlch Gwyn Felsite or the Baron Hill Beds, though they are more likely to be Ordovician than Arvonian. The Felsite has been dated⁷ at 489 ± 13 Myr (= Arenig) and 580 ± 30 Myr (basal breccia). The underlying gneisses have been dated at 565 ± 16 (ref. 7), 562 ± 13 (ref. 8) and 595 ± 12 (ref. 8) Myr, indicating that the 489 ± 13 Myr date for the felsite is the more acceptable. It may, therefore, not be a coincidence that the felsite is in contact with Arenig rocks on the west. Of the Baron Hill Beds little more can be said about their age than that they rest on or are thrust over metamorphosed Gwna rocks (of unknown stratigraphical position—see below) and are supposedly overlain by Ordovician rocks, though the latter are covered by drift¹.

In the Mynachdy and Llanbadrig areas of northern Anglesey, *mélange* rocks mapped as Gwna *Mélange* by Greenly show stratigraphical conformity with the Ordovician or with the Fydyln Felsite, which is considered⁹ to be of Ordovician age. An inclined borehole [SH 302 924]

into these rocks in the Mynachdy area has yielded rare, poorly preserved acritarchs identified as *Cymatigalea*? and *Stelliferidium*? together with a few indeterminate acanthomorphitic forms (S. G. Molyneux, Institute of Geological Sciences, Internal Rep. PDL/81/24). This assemblage suggests that the sampled *mélange* is late Cambrian at the oldest or early Ordovician in age. Greenly's Gwna Group has apparently little stratigraphical significance, including as it does an extensive range of lithologies of widely differing age.

In our reply to Tegerdine *et al.*, we have referred in more detail to the different Ordovician sequences on either side of the proposed fault and to the line of the fault across the Lleyn. The latter does not interfere with the pumpellyite isograd¹⁰ but approximates to its westernmost boundary, and the fault may thus be responsible for the truncation of the saponite zone.

The Cambrian sediments of Pembrokeshire were derived from the south-west and south-east⁵, and it is therefore not surprising that Monian-type fragments are not characteristic of them—wherever Anglesey was at the time.

Our paper showed that the concept of a major dextral displacement along the proposed fault did not originate from the oblique collision model, but from the fundamental geological differences between Anglesey and the mainland. The oblique collision model is invoked as the cause.

It is generally agreed^{11,12} that the Iapetus Suture passes to the south of the Devonian Cheviot lavas. The reference¹³ quoted by Gibbons and Gayer is based on a misreading of the published evidence¹¹.

The quoted faults in Scotland and Eire, apart from being of debatable age and direction of movement¹⁴, occurred within the North Atlantic Plate (north of the Iapetus Suture) and are therefore of limited relevance to events within the southern plate containing the area under discussion. Dextral movements on faults in the Lleyn parallel to the proposed transcurrent fault have been indicated by Tremlett¹⁵ and even by Gibbons¹⁶.

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NUTT AND SMITH¹ suggest that the Menai Straits, North Wales, mark the position of a fault of major regional importance and believe that they can assign >100 km of dextral strike-slip movement to it. The possibility of transcurrent faulting has also been mentioned by Barber and Max².

It is surprising that the geophysical evidence³, which strongly favours the existence of a major fault, was not mentioned by Nutt and Smith. South-east of the proposed fault is a striking correspondence between gravity anomaly contours and geological structure, although the anticlines (Bangor–Padarn Ridge and Harlech Dome) correspond to gravity lows. This suggests density inversion, with a large thickness (0.5–1 km) of less dense rocks, probably Arvonian volcanics and granite, underlying the Lower Palaeozoic. (The suggestion

Matters Arising

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of Rast³ that the gravity anomalies result from basic igneous intrusions in the synclines cannot explain the good fit of gravity isogals with the outcrop pattern, especially around the Harlech Dome.) North-west of the fault, in Anglesey, there is again a good fit with the structure, but without density inversion; a linear positive anomaly of about 45 mGal runs along the Monian amphibolites of the Aethwy Block and similar gravity highs coincide with the other Monian basement blocks of the Central Region and Pentraeth Inliers. However, in contrast to the south-east side of the fault, there is no gravity evidence for any Arvonian, which may be present, but is certainly very much thinner than to the south-east. In contrast to the effect of possible Arvonian granite in Caernarvonshire, there is no gravity anomaly on the published gravity map⁴ in the position of the Coedana Granite in Anglesey. This is surprising in view of its

apparent intrusion into the dense amphibolite basement, and suggests that the granite does not extend to any great depth.

The direction of movement of the Menai Fault might be suggested by the curvature in the strike of the foliation in the Monian to the south-west of Beaumaris in Anglesey. The mylonitic foliation itself may be related to movements on the fault². Later movement seems to have caused a bend in the foliation about an axis dipping ~22° to 084°N. This would suggest oblique slip, with a large component of dip-slip movement raising the north-west (Anglesey) side. The smaller, strike-slip component of movement would suggest a dextral displacement on a map, although this is not in accordance with the apparently sinistral displacement of lithologies in this area on the published geological map.

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NUTT AND SMITH REPLY—Whilst we consulted gravity and aeromagnetic anomaly maps of the relevant areas to make sure that they did not contradict the transcurrent fault hypothesis, lack of space prevented us from discussing these aspects. We therefore thank Kohnstamm and Mann for raising the matter in support of the hypothesis, and would in general agree with their interpretation of the geophysical evidence. A similar interpretation to that of the Anglesey/mainland situation can also be applied across the proposed fault in the Lleyn¹, and, as we have mentioned in reply to Tegerdine *et al.*, the aeromagnetic map² supports the location of the fault along the Dent Line.

The evidence supplied by distortion of the foliation in the Monian should be treated with caution, for it is largely attributable to later and therefore irrelevant tectonic events.

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BOOK REVIEWS

Mending cracks in the nuclear establishment

Alvin M. Weinberg

SIR Alan Cottrell has written a well-balanced, popular account of the issues around which the nuclear energy debate swirls: reactor safety, waste disposal, proliferation, terrorism and the political implications of nuclear energy. He ignores costs, availability of uranium and the need for electricity, for "in the end it is [safety], not any of these other factors, which will eventually decide the acceptability of nuclear power in Western democratic society". I agree with Sir Alan's assessment, though the bombing of the Iraqi reactor at Tamuz could give to the proliferation issue more political force than it now has.

Sir Alan is strongly pro-nuclear, but this does not prevent him from focusing upon his concerns about reactor safety. Though he believes that reactors *can* in principle be made safe enough, he does not say whether they *will* be made safe enough. For Sir Alan, not all reactors are equally safe: brittle fracture of the pressure vessel in a light water reactor remains its Achilles heel, despite the more optimistic conclusions of the 1976 inquiry, led by Walter Marshall, into the integrity of light water pressure vessels. The Marshall Report insisted that such vessels were safe, especially since cracks large enough to be threatening could be detected by ultrasonic probes with more than 95 per cent probability.

Ninety-five per cent reliability is surely a difficult — some would say impossible — goal. Sir Alan invokes the somewhat controversial 1979 OECD report to conclude that only one-half, rather than 95 per cent, of all serious cracks can be detected: "an improvement is needed . . . up at least to the Marshall figure". Without quite saying so, Sir Alan still seems to be sceptical of light water reactors.

Yet his position is not impregnable. In requiring a 95 per cent instead of 50 per cent detection probability, he in effect accepts a brittle failure probability of 10^{-7} per year, but rejects a probability of 10^{-6} per year. Such fine tuning seems to me artificial. More realistic than such precise computation of extremely unlikely events is the assurance that the failure of a pressure vessel is all but impossible: both 10^{-6} and 10^{-7} per year are "all but impossible".

Sir Alan's criterion of acceptability follows from a too literal application of

How Safe is Nuclear Energy? By Alan Cottrell. Pp.124. ISBN 0-435-54175-7. (Heinemann Educational: 1981.) £2.50, \$6.



Sir Alan Cottrell — his concern about the embrittlement of pressure vessels is open to question but nonetheless should be taken seriously.

Farmer's recommendation of a safety standard:

$(\text{curies released in an accident}) \times (\text{probability of accident}) < 1.$

But how many curies might be released in an accident is by no means as clear as Sir Alan assumes. At Three Mile Island about 15 Ci, that is, less than 10^{-6} of the inventory, was actually released. Apparently, in the presence of water the iodine remains in solution rather than becoming airborne. This is an inherent safety advantage of the light water reactor, though whether one can count on the mechanism in the event of a catastrophic pressure vessel failure is debatable. Should these estimates prove correct, water-cooled reactors would pose at the very worst a much smaller risk to the public than Sir Alan estimates.

Yet neither can Sir Alan's concerns be ignored. The US Nuclear Regulatory Commission (NRC) has ordered that 14 of the older light water reactors be re-analysed to determine whether radiation has so

embrittled the pressure vessels as to constitute a significant hazard in the event of an emergency injection of cold water. The action of the NRC demonstrates that the issue of pressure vessel embrittlement will not go away easily, even if one disagrees with Sir Alan's specific, and somewhat rigid, criterion of acceptability.

Are some reactor types *inherently* more "forgiving" than others? Until the United Kingdom debate on light water reactors versus advanced gas-cooled reactors, and before Three Mile Island, this question was hardly raised: all reactors were equally safe, if not by their nature, then by their design. The current widespread nuclear moratorium on new reactors might be a time to re-examine the relative forgiveness of different reactor types.

It is not a foregone conclusion that gas-cooled reactors will win in this competition. Copper-free steels that are much less subject to radiation embrittlement than were the older, less pure materials, now go into pressure vessels for newer light water reactors. This, together with the small release of iodine from water reactors (provided some liquid water remains after an accident), could balance the advantages of high heat capacity, low power density and pre-stressed concrete pressure envelopes of gas-cooled reactors.

When in 1946 the Oak Ridge group persuaded the then Captain Rickover to adopt pressurized water for *Nautilus*, radiation embrittlement of pressure vessels was not recognized as a concern. It is therefore not too surprising that 35 years later the issue of pressure vessel embrittlement has come alive. To be sure, the newer steels, plus the better inspection techniques and the lower iodine release fraction, taken together ought to allay Sir Alan's as well as the public's concern. But I hope we use the pause in deployment of reactors, especially in the United States, to ask whether the line of development we launched upon 35 years ago is still the path we ought to be following in a second nuclear era. Though Sir Alan's book will annoy both anti-nukes and most of the nuclear establishment (which is committed to light water reactors), his concerns about fracture of pressure vessels must be taken seriously. □

Alvin M. Weinberg was formerly Director of the Oak Ridge National Laboratory, Oak Ridge, Tennessee. In 1946, together with F.H. Murray, he proposed the adoption of the pressurized water reactor.

Chemical phrenology of the hypothalamus: another illusion?

George Fink

Handbook of the Hypothalamus. Edited by Peter J. Morgane and Jaak Panksepp. Vol. 1 *Anatomy of the Hypothalamus*, pp.756, ISBN 0-8247-6834-5; Vol. 2 *Physiology of the Hypothalamus*, pp.688, ISBN 0-8247-6881-7. (Dekker: 1979–1980.) \$145 per volume.

"It is quite curious that psychologists, after so many years of seeing the remainder of the brain only as an appendage of the hypothalamus, suddenly now see each complex behaviour as being 'generated' by a particular chemical pathway", writes Peter Morgane, one of the editors of the *Handbook of the Hypothalamus*, in a swinging attack on the concept of brain centres and the new chemical phrenology. However, neither the introductory chapter in Vol. 1 by Morgane nor the other chapters on anatomy provide a realistic alternative. We are still far from a perfect understanding of the precise anatomy of the hypothalamus, but even if we were approaching this goal, would we be any closer to the "functional truth"? Thus, for example, although the intrinsic and extrinsic connections of the cerebellum are almost perfectly understood — certainly better than those of the hypothalamus — we are still not able to state precisely how the cerebellum controls movement.

Several of the chapters in Vol. 1 are too long, and dwell tediously on detail which is neither new, exciting nor especially relevant to current research on hypothalamic structure or function. The development of the hypothalamus, for example, is the subject of a scholarly contribution by Keyser, in which emphasis is placed on classical embryological issues such as the relationship between the sulcus limitans and the hypothalamic sulcus, but, surprisingly, no mention is made of either the development of specific neurotransmitter systems or of the critical periods of development related, say, to sexual differentiation of the brain. In a 110-page chapter, Ambach and Palkovits give the most detailed and beautifully illustrated description of the vasculature of the hypothalamus that, I think, has been published in the present century, but do not include, for example, either quantitative data on the density of capillaries relative to perikarya or a discussion of endothelial-glial-neuronal relationships.

The chapters on the neural connections

of the hypothalamus by Palkovits and Zaborsky, and on the medial hypothalamus by Renaud, do, however, contain useful information presented in handbook style. Millhouse's beautifully illustrated and thorough account of Golgi anatomy also provides a useful starting point for the workers who will eventually settle down to unravel definitively the intrinsic connections and cyto-architecture of the hypothalamus. Parent's contribution on monoaminergic and cholinergic neurons will be especially valuable to those doing research on fish, amphibia and reptiles, and Sutin and McBride re-examine hypothalamic limbic-brain-stem connections mainly with horseradish peroxidase and electrophysiological techniques. The editors have also included a "cyto-architectonic atlas of the hypothalamus", but this might have been more useful had the coronal sections been related to stereotaxic co-ordinates and had the descriptions of cells been more quantitative and detailed.

Vol. 2 on physiology starts with a competent chapter by McKelvy and associates on the biosynthesis of peptides, which also bravely tackles the interesting story of why and how several respected workers were led to think for a time that thyrotropin releasing hormone was synthesized extra-ribosomally. This chapter might have been even better had McKelvy not included the rushed catalogue, at the end, of facts about non-peptide transmitters. Knigge and his colleagues then detail the distribution of some of the peptide-containing perikarya, display some fascinating scanning electronmicrographs of the third ventricle and continue their polemic on the importance of the tanocytes in transporting substances from the third ventricle to the hypophyseal portal vessels. One major and at least two minor errors of scholarship detract from the standard of this chapter. The major error occurs in Table 3 (p.77), which implies that vasopressin and oxytocin are selectively located in the supraoptic and paraventricular nuclei, respectively; the minor errors are that it was Brown-Grant, in the early 1960s, who developed the idea of selective thyroxine uptake by the median eminence and this should have been acknowledged in Fig. 49 (perhaps the most important diagram in this chapter), and Popa and Fielding did not work at Oxford (p.102).

Then follow standard, but nonetheless sound, accounts of the hypothalamic regulatory hormones (by Vale and associates) and the neurotransmitter (classical, non-peptide) regulation of anterior pituitary function (by Kordon and associates). De Wied and Witter describe in detail the way in which oligopeptides affect behaviour, mainly in animals and under

strictly defined experimental conditions. These authors probably over-reach themselves in areas of physiology as shown, for example, by the confused statement on the relevance of protein synthesis to the action of luteinizing hormone releasing hormone (p.361). Spector throws a timely challenge at the Bernard-Cannon concept of homeostasis ("Every living organism, from birth to death, is in a state of non-equilibrium"), and then explores the involvement of the hypothalamus in the immune response and cancer in the context of his (Spector's) theory of the "fluctuating central state". Spector's review of the experimental evidence is especially important since several recent clinical studies suggest that the mental state, by way of a neuro-endocrine mechanism, may either predispose the individual to and/or affect the course of systemic disease. A succinct chapter by Frohman on the control of metabolism by the hypothalamus concludes with the mid-1970s view (not that it has been replaced by any better view) of anorexia nervosa and obesity. Frohman's chapter is complemented by Oomura's account of the effects of metabolites on neuronal activity measured by single and multiple unit recording.

Overall, the standard of this work is quite variable and in some technical respects, such as the reproduction of micrographs and electronmicrographs, is uniformly poor. Too many of the chapters are written as standard reviews, and some are only slightly modified versions of accounts published in the many other books on the hypothalamus that have recently appeared. The work could have been made much more valuable had an attempt been made to tabulate data relevant to current research. For example, there are no comprehensive tables showing quantitative data on the distribution of transmitters and the enzymes that synthesize or metabolize non-peptide transmitters, the distribution of the various types of neurons, or the electrophysiological characteristics of neurons in various nuclei. To obtain these facts, the reader must wade through a lengthy text and often look up references cited by the authors — the point of a "handbook" is to reduce this laborious task to a minimum.

However, although these volumes are less than a handbook should be, the novel treatment of some of the data, together with the extensive bibliography (mostly up to 1977–1979), probably makes it reasonable for the aspiring or practising chemical phrenologist to recommend the work to the nearest main library. □

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● Volumes 3A and 3B of the *Handbook of the Hypothalamus*, dealing with behavioural studies, have been published recently and will be reviewed in *Nature* at a later date.

● The paperback edition of *Wholeness and the Implicate Order* by David Bohm (reviewed in *Nature* 291, 435) is published today in the United Kingdom by Routledge & Kegan Paul, price £3.95.

Transposition: from disbelief, to confirmation, to implications

J.R.S. Fincham

Movable Genetic Elements. Cold Spring Harbor Symposia on Quantitative Biology, Vol. XLV, Parts 1 and 2. Pp. 1,025. ISBN 0-87969-044-5. (Cold Spring Harbor Laboratory: 1981.) \$130 (US), \$156 (elsewhere).

IN THE Cold Spring Harbor Symposium of 1951, Barbara McClintock presented the first fairly full account of her data and ideas on "controlling elements" in maize. Her message can, with hindsight, be regarded as two-fold — first, that the maize genome contains elements capable of frequent transposition and, second, that such transpositions are likely to play an essential role in normal cellular differentiation. Even though it was the custom at the time to publish verbatim the discussion following each symposium contribution, no discussion at all is recorded after McClintock's paper. The reactions, or so one has heard from those who were present, mostly ranged from incomprehension to disbelief.

Barbara McClintock did not contribute to the 1980 symposium, but she was present and must have been gratified to hear the ample confirmation, compressed here into two tightly packed volumes, of her main concepts. In one respect only is her original picture apparently falsified. Whereas she linked the kind of genetic movement which she saw in maize with controlled cell differentiation, it now appears that there are two distinct kinds of phenomenon.

First, there are the almost randomly moving, arguably "selfish", sequences. These are still not characterized at the molecular level in maize (though this will probably not remain true for much longer), but a number of examples of what are almost certainly closely comparable DNA elements are known in two other eukaryotes, yeast and *Drosophila*. In *Drosophila*, indeed, so many have now been detected that one begins to feel the need for a proper taxonomy and system of nomenclature. Movable elements like these will probably turn out to be present in all organisms. They are not, to be sure, totally anarchic. Most of the time their replication and movement is held in reasonable check, sometimes by repressors which they code for themselves — what one might call molecular self-restraint. But they seem likely to be significant mainly as generators of genetic noise. The future exploration of their relationships with their host genomes will constitute almost a new subject — intra-organism ecological genetics. As one group of papers makes plain, they bear an uncanny resemblance to the genomes of the retroviruses of mammals and birds.

Controlled and *specific* DNA changes are also well represented in these volumes, but as a totally separate set of cases. Pride of place must be given to the rearrange-

ments of mammalian antibody genes during the differentiation of antibody-producing cells. The 1980 state of play in this sensationally developing field was thoroughly aired in the symposium. More information is now available pointing to the mutational origin of at least much of the observed antibody diversity. But the main elements of the current picture are laid out here, including the mechanism of heavy-chain switching by DNA deletion and the production of both membrane-bound and secreted antibody by different modes of splicing at the RNA level (a way of diversifying gene products of which much more is being and will be heard).

Whether immunity is unique among vertebrate systems of irreversible cellular differentiation in requiring DNA rearrangement is not known; many of us are waiting or searching for the next case. It is, however, already clear that, among prokaryotes and unicellular eukaryotes,

controlled inversions or transpositions of DNA segments are an important means of *reversible* cell differentiation. Yeast mating-type switching, documented here in great detail, is the best eukaryote example so far, but two papers on antigen variation in trypanosomes give promise of a second example of comparable fascination and greater practical importance.

Nearly all of the leading workers on movable DNA elements are represented in these volumes, including the Russian drosophilists who were, unfortunately, unable to be present in person. The production is up to the usual high Cold Spring Harbor standards. All of the Cold Spring Harbor Symposia have been good buys and many have been landmarks. This one ranks among the very best of them. □

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Face to face with the disposable computer

Simon Lavington

Minicomputers: A Reference Book for Engineers, Scientists and Managers. Edited by Y. Paker. Pp. 505. ISBN 0-85626-188-2. (Abacus Press: 1981.) £32.50. Available in the US from Heyden & Son, \$70. **Interfacing Microcomputers to the Real World.** By Murray Sargent III and Richard L. Shoemaker. Pp. 288. ISBN pbk 0-201-06879-6. (Addison-Wesley: 1981.) \$14.50, £8.70.

COMPUTERS are now firmly part of the grammar of science. It is not just that programming has become a commonplace activity; the incorporation of small computers into experimental apparatus is now within the capability and budget of most scientists. With the advent of programmable electronics — microprocessors and their related chip sets — there are few limits to what the enthusiast can do with small computers. Even the traditional experts, the computer science academics, the pompous data processing managers, the loquacious salesmen, are at last being circumvented by ordinary mortals who are willing to "have a go". Of crucial importance to the non-specialist is how to connect his equipment to a computer, or his own computer to a large one. The umbrella term "interfacing" covers such topics. Hardware and software techniques for interfacing are the principal themes of both the books under review, and they both tackle them in the context of small computer systems.

The book by Sargent and Shoemaker is typical of the new movement amongst computer users. It swings along with an

informality which refuses to be slowed down by the odd gap in the authors' technical knowledge. It is a cook-book of tricks for getting the most out of a Z80 microprocessor and a handful of TTL chips, written by two professors of optical science who have obviously had a lot of fun doing just that. But from this springs its drawback also: if you do not already have at least a modest electronics background and an enthusiasm for assembler programming, the book is nothing but gibberish.

In contrast, the collection of papers assembled by Dr Paker is written by experts. It looks at the general rather more than the particular, and is in the best traditions of textbook turgidity. Its big drawback is that the material derives from courses of lectures given some years ago and, though claiming to be "updated, re-edited and condensed", shows its age in some of the technical details. Fortunately, many of the principles expounded transcend the fleeting years and the book could find a respectable home in the reference section of a library. It is best regarded as a series of review papers with special reference to minicomputers in real time and process control. The chapter on the CAMAC standard interface is particularly useful.

How is it possible that two books, both dealing with interfacing to small computers, should contain so much mutually exclusive material? Apart from the different time-references of the material covered, there is a more subtle reason which relates to the antecedents of

minicomputers and microcomputers. Minicomputers were a downwards development from large, general-purpose, stored-program machines, whereas microcomputers were an upwards development from discrete electronics components. Both minis and micros retain the technical flavour of their respective forebears, together with a contrasting aura of formality versus informality, structure versus improvisation. Microprocessors and their related chip sets have become sufficiently cheap for improvisation to be, if not academically respectable, then at least cost-effective in terms of time and effort. Furthermore, the capability for ingenious local control now offered by a microprocessor is surely the answer to every experimental scientist's prayer. Chips such as the Z80 ought to be as commonly and as casually used as the bunsen burner of yesteryear.

It is in this spirit that the book by Sargent and Shoemaker should be read. It is unashamedly biased towards the widely-used Z80 family of microprocessors, with the TRS-80 system predominating. Practical circuits and assembly-code sequences are given for dealing with AC control, A/D/A conversion, signal averaging, stepper motors, displays etc., and serial communication using a variety of physical media. There is an intriguing series of 14 step-by-step experiments (hardware and software) for the enthusiast armed with a Z80 starter kit. This book, like the Z80, should be bought, used well and then disposed of when it becomes obsolete. □

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mass with velocity was not a theory of electromagnetic mass alone, but of all mass. Miller gives a good picture of this situation, in which special relativity, as a theory in its own right, had to wait about five years for widespread acceptance.

On a couple of points of detail, I was sorry that Miller (p.134) repeated the old myth that Boltzmann's constant and Avogadro's number were not reliably known before 1905 (or, presumably, until they could be inferred from Millikan's precise measurements of e around 1915). In 1900 Planck published values of these quantities differing by less than 3 per cent from today's. Also, I was puzzled by Miller's assertion (p. 266) that the Ives-Stilwell experiment of 1938 remains the only positive proof of time dilation. Surely the Hafele-Keating experiment (1971), with atomic clocks carried around the world in opposite directions, was as direct a demonstration as one could wish for.

... and then came Einstein ...

A.P. French

Albert Einstein's Special Theory of Relativity: Emergence (1905) and Early Interpretation (1905-1911). By Arthur I. Miller. Pp.466. ISBN hbk 0-201-04680-6; ISBN pbk 0-201-04679-2. (Addison-Wesley: 1981.) Hbk \$39.50, £20.10; pbk \$27.50, £13.45.

PROFESSOR Miller has undertaken an interesting and worthwhile task in this detailed study of the historical background and the initial reception of Einstein's special relativity theory, and in particular of his epoch-making first paper on this subject in 1905. In his introduction, Miller makes the telling point that:

... it is difficult to imagine a teacher of English who has never read one of Shakespeare's plays. But few people today, including physics researchers, teachers of physical science or philosophers of science, have carefully read Einstein's relativity paper of 1905 ...

— this despite its immense impact, not only in its particular field, but also on the whole philosophy of theoretical science. Miller also remarks that:

... While many in-depth analyses of the works of high literature are available to humanistic scholars, physicists have virtually no access to analyses that guide the reader through the real and apparent complexities of a major scientific work, placing the work in its proper historic context ...

It has been Miller's aim to provide within a single book a detailed exegesis of the 1905 paper, preceded by a full account of the developing situation in electromagnetic theory during the 15 years prior to its appearance. In a lengthy chapter which occupies over a quarter of the book, he takes the reader through the complex

history of electrodynamics after Maxwell, as developed chiefly by Hertz, Lorentz, Abraham and Poincaré. At first, the problem was to reconcile an ether-based electromagnetic theory with the optical phenomena observed by Fizeau and Michelson. However, after the discovery of the electron in 1896/7, the quest began for an electrical theory of matter, including various specific theoretical expressions for the variation of electron mass with velocity — predictions that were diligently compared with the contemporaneous experimental findings of Kaufmann.

Then came Einstein. Miller's reminder of the complexity and artificiality into which classical electrodynamics had been driven helps one to appreciate even better the magnitude of Einstein's genius. Reading the opening sections of his 1905 paper is like going into a side room for a quiet and thoughtful conversation after being at a noisy, crowded cocktail party. His profound and deceptively simple insights led, as we all know, to a straightforward, exact and complete explanation of all the phenomena that had so exercised the experts. Miller conducts us through Einstein's paper, section by section, with appropriate reference to relevant contemporary work and the reactions of other physicists. Two features of these reactions seem to stand out. One is the failure of the world of physics in 1905 to recognize or acknowledge the conceptual gulf between Einstein's approach and the ether-based theories of Lorentz. The other is a similar failure to see that Einstein's results, based as they were on fundamental concepts of space and time, transcended the limits of electrodynamics in particular. Thus, for example, his formula for the variation of



I have a further, more substantial criticism of Miller's treatment. He quotes what Einstein once said about Mach's deep insights into the development of mechanics, even where specific knowledge of what the early workers thought or did was lacking. Miller seems to try to emulate this in the case of Einstein. In particular, with regard to Kaufmann's measurements of electron mass as a function of velocity, Miller suggests that Einstein knew of these results in 1905, but failed to mention them because they conflicted with the predictions of his theory. This seems to me entirely out of character for Einstein, and represents an unwarranted aspersion on his scientific integrity. Conjectures of this sort can only detract from an otherwise scholarly discussion. It seems far more likely that, just as in his development of the photoelectric equation, Einstein did not know or care very much about the imperfect experimental data; the foundations of his theorizing were much deeper and stronger. Except for this criticism, however, I would recommend Miller's book to anyone who wishes to find a detailed picture of the antecedents and birth of special relativity. □

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